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**ASPECTS OF RESPIRATORY FUNCTION DURING EXERCISE IN THE  
THOROUGHBRED HORSE IN HEALTH AND DISEASE**

<sup>DRIAN</sup>  
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**A thesis submitted in partial fulfilment of the  
requirements of the Open University for the  
degree of Doctor of Philosophy**

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## ABSTRACT

The horse's enormous respiratory reserve enables it to increase the respiratory load on the lungs many fold during exercise, hence low-grade respiratory disease is difficult to detect at rest, although of vital importance during heavy exercise. Diagnostic exercise testing is, therefore, of great interest and this thesis describes its application to the investigation of respiratory disease in the Thoroughbred horse.

Horses were exercised on a treadmill whilst respiratory flow rates were recorded using ultrasound flow transducers, respired gas concentrations measured by mass spectrometry and blood gas analysis performed. The exercise test involved a warm up followed by two minute canters/gallops at increasing workloads separated by ten minute walks. Validation studies confirmed the suitability of the methods for these studies. A steady state for respiratory variables was reached by ninety seconds of canter/gallop.

The effects of training on exercising respiratory function were investigated by exercise testing horses following a sedentary period and after a fifteen week training programme. State of training did not complicate clinical interpretation of some variables, e.g tidal volume, respiratory frequency and minute ventilation, whilst peak flow rates were stable at the highest workloads. Arterial oxygen tension and blood pH during exercise were affected by training but other blood gas variables and end-tidal gas tensions were not.

The effect of influenza on respiratory function was studied by challenging partially-immune horses with H3N8 influenza, inducing infection similar to that seen in vaccinated racehorses. Twenty one days after infection most horses showed no changes

in respiratory function but 2/9 horses altered breathing strategy during canter and another showed altered acid-base and blood lactate responses.

Horses with low-grade respiratory disorders showed differences in respiratory function to the experimental animals but the wide normal range of pulmonary function tests limited their one-off diagnostic value. Serial testing may prove to be a more sensitive method.



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## LIST OF ABBREVIATIONS USED IN THIS THESIS

AST	aspartate aminotransferase
$C_aO_2$	arterial oxygen content
$C_{(a-\bar{v})}O_2$	arterial-mixed venous oxygen content difference
$C_{\bar{v}}O_2$	mixed venous oxygen content
CMI	cell mediated immunity
CPK	creatine phosphokinase
C8	canter at 8 m.s <sup>-1</sup>
C10	canter at 10 m.s <sup>-1</sup>
C12	canter at 12 m.s <sup>-1</sup>
C12+3	canter at 12 m.s <sup>-1</sup> on a 3° incline
$f_b$	frequency of breathing
$f_s$	stride rate
GGT	gamma glutamyl transferase
HI	haemagglutination inhibition
[Hb]	haemoglobin concentration
H3N8	haemagglutinin 3 neuraminidase 8 subtype of equine influenza (A/equine-2)
H7N7	haemagglutinin 7 neuraminidase 7 subtype of equine influenza (A/equine-1)
MIF	mean inspiratory flow rate
$P_{(A-a)}O_2$	alveolar-arterial oxygen tension difference
$P_aCO_2$	arterial blood carbon dioxide tension
$P_{(a-ET)}CO_2$	arterial-end-tidal carbon dioxide tension
$P_AO_2$	alveolar oxygen tension

$P_{aO_2}$	arterial blood oxygen tension
$P_ECO_2$	mixed expired gas carbon dioxide tension
PEF	peak expiratory flow rate
$P_{EO_2}$	mixed expired gas oxygen tension
$P_{(ET-a)O_2}$	end tidal-arterial oxygen tension difference
$P_{ETCO_2}$	end tidal gas carbon dioxide tension
$P_{ETO_2}$	end tidal gas oxygen tension
$pH_a$	arterial blood pH
$pH_{\bar{v}}$	mixed venous blood pH
PIF	peak inspiratory flow rate
$PO_2$	oxygen tension
PV	plasma viscosity
$P_{\bar{v}}CO_2$	mixed venous blood carbon dioxide tension
$P_{\bar{v}}O_2$	mixed venous blood oxygen tension
$\dot{Q}$	cardiac output
$\dot{Q}_{max}$	maximum cardiac output
$S_aO_2$	arterial blood haemoglobin saturation
SET	standardised exercise test
SRH	single radial haemolysis
$S_{\bar{v}}O_2$	mixed venous blood haemoglobin saturation
SV	stroke volume
$t_e$	expiratory time
$t_i$	inspiratory time
$t_{re}$	rectal temperature

$t_{\bar{v}}$	mixed venous temperature
TW	tracheal wash
$\dot{V}_A$	alveolar ventilation
$\dot{V}_A/\dot{Q}$	ventilation/perfusion ratio
$\dot{V}_{CO_2}$	carbon dioxide production
$V_d$	respiratory dead space
$V_{dphys}$	physiological respiratory dead space
$\dot{V}_e$	minute volume
$V_i$	inspiratory volume
$\dot{V}_{O_2}$	oxygen consumption
$\dot{V}_{O_{2max}}$	maximum oxygen consumption
$V_t$	tidal volume
V1	virus challenge study 1
V2	virus challenge study 2
V3	virus challenge study 3
$W_{rm}$	mechanical work of breathing

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wished to take part in these studies but allowed themselves to be used with the generosity which man has come to take so much for granted from the horse. I am pleased to say that none of them suffered any serious or long-term injuries as a result of their endeavours.

The studies described in this thesis represent my own work unless otherwise indicated in the text and with these qualifications. Routine haematological and virological assays and tracheal wash analysis were performed by the commercial laboratories of the Animal Health Trust. During the running of the exercise tests samples were run through the blood gas analyser by colleagues in the Department of Physiology since I was involved in running the exercise tests and obtaining samples.

## **PREFACE**

In his classic treatise on horsemanship, Xenophon wrote: 'In the horse, as in the man, all diseases are easier to cure at the start than after they have become chronic and have been wrongly diagnosed'. Although they were written over twenty three centuries ago, these words are still as true today.

Clinicians are becoming increasingly aware that respiratory dysfunction in the racehorse may be an important cause of wastage and reduced performance (Thomson, 1990). There is considerable concern as to the effects of respiratory viral infections on the racehorse, both in the acute phase and as a longer term cause of impaired respiratory function. Subjecting horses which have not fully recovered from the effects of respiratory infections to hard exercise may delay recovery and has been reported to cause myocarditis (Gerber, 1970). Thus it is of great importance to the equine clinician to be able to quantify the duration and effects of respiratory disease on the horse in order to minimise its impact.

The diagnosis of sub-clinical respiratory disease however, presents the equine clinician with a major diagnostic challenge. The resting animal has a considerable reserve respiratory capacity, as a result of which significant lung dysfunction may be present without being detectable upon routine clinical examination. Additionally, in the horse, many tests of pulmonary function which are applicable to the human patient are inappropriate due to the requirement for patient cooperation.

With this in mind, the aims of this thesis are to aid in the characterisation of the effects of

low-grade respiratory dysfunction on the horse and to evaluate certain methods of measurement of respiratory function for their suitability in equine diagnosis.

*For Cora and Sasha*

## CHAPTER ONE - INTRODUCTION

Thoroughbred racing is one of the major leisure industries in the country, with attendance figures for the 1,103 race meetings held in 1990 approaching five million. In that year, over twelve thousand horses in training competed for total prize money of around forty million pounds. The industry is a major employer with around 150,000 people owing their employment either directly or indirectly to racing.

Respiratory disorders form an important cause of wastage to the racing industry. In a survey of wastage in the Thoroughbred racehorse up to the age of four years, Jeffcott, Rosedale, Freestone, Frank, and Towers-Clark (1982) found the major causes of 'lost racing performance to be lameness and respiratory disease'. These authors reported that in four stables in which there were outbreaks of respiratory viral infections during the survey, a number of animals were so affected that they had to be rested for up to six months following infection and even then did not return to their former level of performance.

The importance of respiratory disorders as a cause of lost training time was confirmed by Evans (1988) who found respiratory disease to be the second most common cause of wastage, responsible for 10.4% and 34.3% of lost time in training in 1984 and 1985 respectively. Additionally, subclinical respiratory disease is considered to be an important contributor to the 'loss of performance' syndrome (Mumford and Rosedale, 1980).

A number of respiratory viruses are capable of causing disease in the horse, including equine influenza and equine herpes viruses 1 and 4, as well as certain picornaviruses

(Mumford and Rosedale, 1980). Epizootics of equine influenza have disrupted racing in this country on many occasions (Beveridge, Mahaffey and Rose, 1965). In uncomplicated cases of influenza, recovery occurs within two to three weeks, however there are many reports of horses taking several months to recover and in some cases never again regaining their previous level of performance (Gerber, 1970; Jeffcott *et al.*, 1982; Coggins, 1991; Livesay, 1991). In many cases there are no readily detectable reasons for these horses failing to recover and routine clinical examination of such patients is usually unrewarding. A further possible complication of respiratory viral infections is the development of chronic obstructive pulmonary disease (COPD) (Gerber, 1973).

This thesis tests the hypothesis that low-grade chronic respiratory disease can be a significant cause of reduced respiratory function in the Thoroughbred horse and further that such disease is a possible sequel to equine influenza.

Aspects of respiratory function have been tested during exercise in normal animals at varying stages of fitness, in horses following challenge with the A/equi-2 (H3N8) subtype of equine influenza and in a group of animals referred for the investigation of performance problems in which the history suggested that it was likely that respiratory disease was implicated. Exercise testing was used in order to force utilisation of the reserve respiratory capacity so that low-grade pulmonary dysfunction, undetectable in the resting subject, might become apparent (Denison *et al.*, 1984; West, 1992 ).

To set this study in context it is necessary to review previous work in this field. This is done in the next Chapter.

## **CHAPTER TWO - REVIEW OF PREVIOUS WORK**

### **(i) Common Clinical Experiences**

Respiratory diseases have been recognised in the horse since antiquity, such disorders being described by writers including Aristoteles in 333 BC. There is evidence that epidemics of respiratory infections similar to equine influenza have been a source of economic loss to the horse owner since at least the tenth century AD (Baker, 1986). Certainly a clinical condition known as equine influenza was recognised in the sixteenth and seventeenth centuries (Fleming, 1897).

In the eighteenth and nineteenth centuries, the large numbers of horses kept in crowded conditions in towns and cities meant that the incidence of respiratory infections was high. The King's farrier for Scotland, James Clark drew attention to the fact that horses living outdoors suffered far less from respiratory conditions than housed animals (Clark, 1790) and the application of this finding in the form of increased ventilation and air space for housed horses led to dramatic reductions in the incidence of respiratory disease (Smith, 1905). In France, after 1836, the policy of increasing the air space of housed cavalry horses resulted in their annual mortality rate dropping from twenty to seven per cent (Smith, 1905).

In spite of the lessons learned, even today horses are often kept under managerial conditions which may predispose them to respiratory disease, high stocking rates being combined with inadequate ventilation. Indeed it has been suggested that the incidence of respiratory disease in horses is rising and that this may be associated with modern housing



systems (Sainsbury, 1981). Furthermore, the frequent mixing of young horses at race meetings further predisposes to the spread of respiratory infections.

The importance of respiratory infections to the racing industry may be gauged from the fact that in a survey of wastage amongst racehorses in training in 1984 and 1985, respiratory disease was the second most common cause of horses being unable to be trained, contributing 10.4% and 34.3% of days lost in 1984 and 1985 respectively (Evans, 1988). Of the time lost through respiratory disease, over 89% was reportedly due to viral and bacterial infections.

The term equine influenza was for many years applied indiscriminately to diseases of horses characterised by acute respiratory signs which, in addition to equine influenza probably included conditions caused by other agents such as equine herpesviruses and equine arteritis virus (Beveridge, 1965; Bryans and Gerber, 1972). Equine influenza virus was first isolated in 1956 (Sovinova, Tumova, Pouska and Nemec, 1958), whilst at around this time it was realised that equine viral arteritis and rhinopneumonitis were distinct diseases unrelated to influenza (Doll, 1956). It has since become apparent that other viral agents exist such as the rhinoviruses (Burrows, 1969) which may cause respiratory disease in the horse. Nevertheless equine influenza is an important disease of the horse which has on several occasions this century caused epizootics severe enough to interfere with the British racing programme (Beveridge *et al.*, 1965).

Clinical signs associated with equine influenza have been described by many authors (Waddell, Teigland, and Sigel, 1963; Beveridge, 1965; Gerber, 1970; Bryans and Gerber,

1972; Powell, Thomson, Spooner, Plowright, Burrows and Schild, 1974; Thomson, Mumford, Spooner, Burrows and Powell, 1977; McAllister, 1982; Coggins, 1991). In fully susceptible animals onset of disease may be sudden with spread very rapid. There is frequent coughing and a serous nasal discharge which may later become mucoid. Rectal temperatures may be as high as 41.2°C, with biphasic fever spikes sometimes being seen. There may be low grade submandibular and retropharyngeal lymphadenopathy. Affected animals may be depressed and anorexic, with congestion of the nasal mucosae and elevation of pulse and respiratory rates. Secondary bacterial infections may occur resulting in bronchopneumonia, sinusitis or guttural pouch infections, particularly in young animals. Gerber (1970) stressed that the most important site of infection is the lower respiratory tract and suggested that the condition should not be labelled an upper respiratory tract disease. The same author also suggested that the A/equi-2 subtype (H3N8) is more pneumotropic and more virulent than A/equi-1 (H7N7) strains.

Even though vaccination against equine influenza is now mandatory for horses racing under Jockey Club rules, the recent outbreaks of the disease in the last three years (Livesay, 1991) indicate that it is still an important cause of respiratory dysfunction in the racehorse. In addition to the immediate effects of infection, it has been reported that some animals may take several months to recover and in some cases never regain their previous level of racing ability (Jeffcott *et al.*, 1982; Livesay, 1991). Furthermore there appears to be an association between the occurrence of respiratory viral infections and the development of chronic obstructive pulmonary disease (COPD) in many horses (Gerber, 1973); a similar association between viral respiratory disease and chronic respiratory disease has been reported in man (Witting, Cranford and Glaser, 1959; Rooney and Williams, 1971).

A common clinical presentation for the equine clinician is the horse with the so-called 'poor performance' or 'loss of performance' syndrome. These cases are characterised by a history of unexpectedly poor performance in racing or training and/or prolonged recovery after exercise, with or without the presence of other clinical signs. Low-grade respiratory disease, in particular the effects of respiratory viral infections, is thought to be a major cause of this condition (Mumford and Rossdale, 1980).

## **(ii) Measurement of the respiratory response to exercise**

### *Respiratory flow rates*

Measurements of respiration in exercising horses were performed by Zuntz and Hagemann as long ago as 1898, using stationary equipment alongside horses exercising on a treadmill. More recently, respiration was measured in walking draft animals using mobile equipment (Huxdorff, 1933; Hall and Brody, 1934; Kibler and Brody, 1945; Nadaljak, 1960). Karlsen and Nadaljak (1964) used similar methods on trotting horses pulling sulkies and obtained the first measurements of maximum oxygen consumption ( $\dot{V}O_{2\max}$ ) ( $64.2 \text{ l}\cdot\text{min}^{-1}$ ).

Hörnigke, Ehrlein, Tolkmitt, Nagel, Epple, Decker, Kimmich and Kreuzer (1974a) and Hörnigke, Ehrlein, Tolkmitt, Husch, Nagel, Decker, Epple, Kimmich and Kreuzer (1974b) described a method for measurement of respiratory variables in ridden horses. Respiratory flow rate was measured using a strain gauge pneumotachograph, whilst oxygen tension ( $P_{O_2}$ ) was determined by use of a fast oxygen electrode, both mounted in a facemask. This group reported findings from horses running at speeds up to  $\approx 9.5 \text{ m}\cdot\text{s}^{-1}$  (Meixner, Hörnigke and Ehrlein, 1983; Hörnigke, Meixner and Pollman, 1983), at which maximum flow rates were around  $60 \text{ l}\cdot\text{s}^{-1}$  and oxygen consumption ( $\dot{V}O_2$ ) of the order of  $60 \text{ l}\cdot\text{min}^{-1}$ . The system was initially unsuitable for exercise where respiratory rate ( $f_b$ ) is greater than  $100 \text{ min}^{-1}$ . Although later this method was used to measure flow rates during fast exercise with values for  $f_b$  up to  $148 \text{ min}^{-1}$ , at these speeds the response time of the oxygen electrode was insufficient to determine  $\dot{V}O_2$  (Hörnigke, Weber and Schweiker, 1987). The advent of high speed treadmills has facilitated the measurement of respiratory function during strenuous exercise in the horse and various systems have been used to perform such measurements in horses.

Several authors have reported the use of masks incorporating one way valves, allowing measurement of ventilation volumes (Bisgard, Forster, Byrnes, Stanek, Klein and Manohar, 1978; Persson, Essen and Lindholm, 1980; Thomas and Fregin, 1981; Forster, Pan, Bisgard, Dorsey and Britton, 1984; Pan, Forster, Bisgard, Dorsey and Busch, 1984a and 1984b; Bayly, Schulz, Hodgson and Gollnick, 1987a; Evans and Rose, 1987; Pelletier, Blais, Vrins and Robinson, 1987; Powers, Beadle, Lawler and Thompson, 1987; Rose and Evans, 1987; Evans and Rose, 1988a; Evans and Rose 1988b; Gottlieb-Vedi, Essen-Gustavsson and Persson, 1991). Such equipment generally has a relatively large dead space, some degree of added resistance to breathing on the part of the valves and often lengths of large diameter tubing attached. This type of system may affect ventilation and has been shown to change significantly arterial oxygen tension ( $P_{aO_2}$ ) and arterial carbon dioxide tension ( $P_{aCO_2}$ ) during exercise, the directions of change being downwards and upwards respectively, (Bayly, Schulz, Hodgson and Gollnick, 1987b).

An alternative method involves 'flow through' systems in which a bias flow of ambient air is used to collect expired gas without the use of valves (Seeherman, Taylor, Maloiy and Armstrong, 1981; Bayly, Schulz, Hodgson and Gollnick, 1987a and 1987b; Rose, Hodgson, Kelso, McCutcheon, Reid, Bayly and Gollnick, 1988; Jones, Longworth, Lindholm, Conley, Karas, Kayar and Taylor, 1989a; Hodgson, Rose, Kelso, McCutcheon, Bayly and Gollnick, 1990; Rose, Hendrickson and Knight, 1990; Plummer, Knight, Ray and Rose, 1991; Tyler, Jones, Birks, Pascoe, Steffey, Jarvis, Hinds and Tarkington, 1991; Knight, Sinha and Rose, 1991). These systems are limited in the range of variables that they can measure as they cannot be used to determine respiratory flow rates or, since they provide values averaged over a number of breaths, breath by breath analysis of respiratory

variables. Bayly *et al.* (1987b) found that an airflow rate in excess of 6000 l.min<sup>-1</sup> was necessary to prevent restriction of ventilation (evidenced by a P<sub>a</sub>CO<sub>2</sub> greater than control values with no mask).

Respiratory flow rates have been measured in horses using a Fleisch pneumotachograph and differential pressure transducer (Art and Lekeux, 1988; Art and Lekeux, 1989a and 1989b; Art, Lekeux, Gustin, Desmecht, Amory and Paiva, 1989). Art and Lekeux (1988) found it necessary to use two different sizes of pneumotachographs to record the range of flow rates encountered between 0 and 5 m.s<sup>-1</sup> in ponies. In addition, this system was found to have a significant depressive effect on f<sub>b</sub> during trotting.

Slocombe, Covelli and Bayly (1992) used a mask with paired pneumotachographs on Standardbred horses and found a small, but significant effect of this system on arterial oxygen tension (P<sub>a</sub>O<sub>2</sub>). During galloping, there was build up of respiratory secretions on the pneumotachograph screens which would have caused slight overestimation of air flow rates and tidal volume (V<sub>T</sub>), a common problem with pneumotachographs.

Landgren, Gillespie and Leith (1991) described the use of a pneumotachograph incorporated into a flow through system with large amounts of tubing. Flow rates of 7,200-10,200 l.min<sup>-1</sup> were necessary and the authors felt that 'these substantial breathing loads probably influenced breathing'.

Ultrasonic flow transducers, in which airflow is detected by the change in phase of ultrasound signals passing across the path of flow, have been used to measure respiratory

gas flow in man (Kou, Peickert, Polenske and Busby, 1984; Buess, Pietsch, Guggenbühl and Koller, 1986). Woakes, Butler and Snow (1987) described the use of this method in the horse, using a modification of an instrument originally used on flying geese (Woakes and Butler, 1980; Woakes, 1986). Two transducers mounted in a lightweight mask were used to measure airflow through each nostril whilst respired gas compositions were measured by mass spectrometry.

This system has been used in a number of investigations in the horse (Anderson, Butler, Roberts, Smale, Snow and Woakes, 1989; Anderson, Butler, Snow and Woakes, 1990; Art, Anderson, Woakes, Roberts, Butler, Snow and Lekeux, 1990; Butler, Woakes, Anderson, Smale, Roberts and Snow, 1991; Young, Alexander, Woakes, Butler and Anderson, 1992; Butler, Woakes, Smale, Roberts, Hillidge, Snow and Marlin, 1993a; Butler, Woakes, Anderson, Roberts and Marlin, 1993b). It allows measurement of a wide range of respiratory variables, as well as breath by breath analysis. Additionally the system is lightweight, has low resistance and deadspace and, since slight modification was made to the original system reported by Woakes, Butler and Snow (1987) is fairly resistant to the effects of moisture in expired gas.

### *Respiration and Locomotion*

Various observers have remarked that respiration and locomotion appear to be linked during canter and gallop in the horse and it was suggested by Cook (1965) that the clinician could distinguish inspiration and expiration by observing their relationship to gait. Specht (1965) measured respiratory frequency in exercising horses telemetrically and suggested that a relationship exists between the respiratory cycle and locomotion.

Attenburrow (1971) described a telemetric system to record the sound vibrations produced by respiratory airflow during exercise. By comparing recordings of respiration with footfall data, he confirmed the 1:1 synchrony of respiration with locomotion and described the relationship between various phases of both. Additionally, he noted that inspiratory time during canter and gallop is remarkably constant in normal horses but was markedly altered in two horses with respiratory disorders.

Later reports confirmed a 1:1 frequency relationship between respiration and locomotion (Attenburrow and Flack, 1974; Hörnicke *et al.*, 1974b; Hörnicke and Meixner, 1977; Hörnicke, Meixner and Pollman, 1983). Although the usual relationship between locomotion and respiration is 1:1, Hörnicke *et al.* (1974b) reported a 1:2 ratio in some horses, whilst Frevert, Nations, Seeherman, Loring and Banzett (1990) found some horses who breathed at ratios other than 1:1.

Attenburrow (1982 and 1983) reported that synchronisation of respiration and stride occurs in terms of phase as well as frequency so that inspiration and expiration are related to defined phases of limb movement. He suggested that the events of locomotion during canter and gallop influence respiration by physically driving inspiration and expiration.

Bramble and Carrier (1983) noted that 1:1 synchronisation of locomotion and respiration has been observed in other running mammalian species such as dogs and hares, whilst in man synchrony tends to occur only in experienced runners and usually at higher stride:breath ratios than 1:1. They suggested that the ratio in quadrupeds could be determined by impact loading of the thorax via the forelimbs, by phase differences between



musculoskeletal and abdominal visceral motion (the latter resulting in the so-called 'visceral piston' effect) or by flexion of the back. Although these authors reasoned that tidal volume ( $V_t$ ) may be directly related to stride length during gallop, Anderson *et al.* (1990) showed that cantering horses can, in fact, vary  $V_t$  independently of stride length.

Bramble and Carrier (1983) reported occasional entrainment of locomotion with respiration in trotting horses. Although this finding has not been supported by most other workers, for example Attenburrow and Flack (1974) and Hörnicke, Meixner and Pollman, (1983), the latter group did remark on observations by Karlsen and Brejtse (1965) that horses running in trotting races synchronised respiration with stride at a ratio of 1:1 during sub-maximal exercise, switching to lower ratios (usually 1:2 but sometimes 1:1.5, 1:2.5 or 1:3) at maximum effort.

Young *et al.* (1992) examined records of respiratory flow (obtained using ultrasound flow transducers) together with ciné film of horses cantering at 9 and 11 m.s<sup>-1</sup> on a treadmill, to investigate the relative contributions of the mechanisms proposed by Bramble and Carrier (1983) to the work of respiration. They suggested that back flexion is likely to contribute to driving ventilation, but that the visceral piston and loading of the thorax do not. However, it must be remembered that the horse exercising on a treadmill does not move forward, hence there may be less induced visceral movement resulting in a reduction in the visceral piston effect compared with exercise in the field.

The degree to which stride-related mechanical forces contribute to respiration has been investigated for man (Topulos, Banzett, Reid and Mead, 1988) and the horse (Frevert *et*

*al.*, 1990). It was found that in men running at 2.7-2.9 m.s<sup>-1</sup>, the contribution of stride to  $V_t$  was low (15 ml/step), whilst in the galloping horse the volume change due to striding was at most 10-20% of  $V_t$ .

### *Respiratory gas exchange*

Although Persson described collection of blood samples from trotting horses undergoing treadmill exercise in 1967, until relatively recently most exercise studies involving measurement of blood parameters were carried out by obtaining samples as soon as possible after exercise. Such studies showed that immediately after 'strenuous' exercise ( $\geq 10$  m.s<sup>-1</sup>)  $P_aO_2$  was normal or elevated<sup>1</sup> and  $P_aCO_2$  reduced, whilst arterial and venous (jugular and mixed venous) pH were reduced compared with resting values (Krzywanek, 1974; Milne, 1974; Krzywanek, Milne, Gabel and Smith, 1976; Milne, Skarda, Gabel, Smith and Ault, 1976). Milne (1974) reported little change in mixed venous oxygen and carbon dioxide tensions ( $P_{\bar{v}O_2}$  and  $P_{\bar{v}CO_2}$ ), whilst Milne *et al.* (1976) found an increase in  $P_{\bar{v}CO_2}$  after exercise and Krzywanek *et al.* (1976) reported increases in both  $P_{\bar{v}O_2}$  and  $P_{\bar{v}CO_2}$ .

The studies discussed above were all performed on Standardbred horses performing trotting exercise on tracks with the exception of the work of Milne (1974) which involved ridden horses of various breeds. Blood samples were obtained by direct puncture except for mixed venous samples which were drawn through a previously placed catheter.

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<sup>1</sup> After 'moderate' exercise ( $\approx 5.8$  m.s<sup>-1</sup>)  $P_aO_2$  was reduced in the study of Milne (1974).

Since many respiratory variables alter rapidly in the first minute post-exercise, it is necessary to obtain samples during exercise rather than shortly after it (Fregin and Nicholl, 1977). Various methods for collection of blood samples during exercise in ridden horses have been described, involving either drawing of samples by the rider (Littlejohn and Kruger, 1976; Bayly, Grant, Breeze and Kramer, 1983) or collection via radiotelemetry (de Waal, Littlejohn, Potgieter, van der Berg, Minaar and Smith, 1986, - modified for anaerobic sample collection by Littlejohn and Snow, 1988).

Bergsten (1974) obtained arterial and mixed venous blood from Swedish riding horses trotting on a treadmill at four  $\text{m.s}^{-1}$  and found no change in  $\text{Pao}_2$  and a decrease in  $\text{P}_{\bar{\text{v}}\text{O}_2}$  and  $\text{P}_{\text{a}}\text{CO}_2$  during exercise.  $\text{P}_{\bar{\text{v}}\text{CO}_2}$  was increased after three minutes of exercise but had returned to resting levels by ten minutes. Thomas and Fregin (1989) exercised horses at  $2.28 (\pm 0.06)$   $\text{m.s}^{-1}$  on an 11.5% incline for thirty minutes and found no effect on  $\text{P}_{\text{a}}\text{O}_2$  or  $\text{P}_{\text{a}}\text{CO}_2$ .

During strenuous exercise, Bayly *et al.* (1983) and Thornton, Essen-Gustavsson, Lindholm, McMiken and Persson, (1983) reported the occurrence of arterial hypoxaemia, in Thoroughbred horses during maximum speed ridden exercise over 1,600m in the former study and in Standardbreds undergoing treadmill exercise at  $8.5 \text{ m.s}^{-1}$  on a 10% incline in the latter.  $\text{Pao}_2$  fell from 96.2 mm Hg at rest to 77.4 mm Hg in the Thoroughbreds and from 105.45 to 62.25 mm Hg in the Standardbreds. Arterial hypercapnia developed in most horses in both studies. Thornton *et al.* (1983) also found a fall in  $\text{P}_{\bar{\text{v}}\text{O}_2}$  and a doubling of  $\text{P}_{\bar{\text{v}}\text{CO}_2}$  during exercise, with an immediate change towards resting values upon cessation of work for all blood gas parameters except  $\text{P}_{\bar{\text{v}}\text{CO}_2}$  which returned to resting values more slowly.

Littlejohn and Snow (1988) found that arterial hypoxaemia developed within twenty seconds of the start of exercise in Thoroughbreds ridden at 14 to 15 m.s<sup>-1</sup>. They suggested that this decrease may increase buffering capacity due to the Haldane effect.

The occurrence of exercise-induced arterial hypoxaemia and hypercapnia has since been confirmed by many authors (for example Bayly *et al.*, 1987a; Evans and Rose, 1988a; Evans and Rose, 1988b; Bayly, Hodgson, Schulz, Dempsey and Gollnick, 1989; Anderson *et al.*, 1989; Wagner, Gillespie, Landgren, Fedde, Jones, DeBowes, Pieschl and Erickson, 1989; Art *et al.*, 1990; Butler *et al.*, 1993a). Jones, Taylor, Lindholm, Straub, Longworth and Karas (1989b) found that mixed venous (pulmonary arterial) temperature ( $t_v$ ) rose faster than rectal temperature ( $t_{re}$ ) during exercise and suggested that in studies in which blood gases are corrected to  $t_{re}$  rather than  $t_v$ , the degree of hypoxaemia reported may be falsely high (by as much as a half) and the hypercapnia underestimated. A similar differing rate of response of  $t_{re}$  and  $t_v$  to exercise was shown in ponies by Pan, Forster and Kaminski (1986).

In highly-trained human athletes, exercise-induced arterial hypoxaemia has been reported (Dempsey, Hanson and Henderson, 1984), but without the occurrence of arterial hypercapnia. A widening of the alveolar-arterial  $P_{O_2}$  difference ( $P_{(A-a)O_2}$ ) is seen associated with exercise in both species (Dempsey, Hanson and Henderson, 1984; Hammond, Gale, Kapitan, Ries and Wagner, 1986; Bayly *et al.*, 1987a; Bayly *et al.*, 1989; Wagner *et al.*, 1989).

Bayly *et al.* (1987a) found that during exercise whilst breathing hyperoxic gas mixtures (inspired oxygen fraction: 0.242-0.252), alveolar  $P_{O_2}$  ( $P_{AO_2}$ ) increased and arterial hypoxaemia was largely abolished, however  $P_{(A-a)O_2}$  remained high.

In man, it is believed that the major cause of exercise-induced arterial hypoxaemia is diffusion limitation due to the short transit time of pulmonary capillary blood under such conditions (Dempsey *et al.*, 1984; Torre-Bueno, Wagner, Saltzman, Gale and Moon, 1985). Wagner *et al.* (1989) performed multiple inert gas elimination studies in exercising horses and concluded that the primary cause in this species is also diffusion limitation (accounting for over 75% of the  $P_{(A-a)O_2}$  at high speeds), whilst hypoventilation, evidenced by a higher  $P_{aCO_2}$  than expected, also contributes. Ventilation-perfusion ( $\dot{V}_A/\dot{Q}$ ) inequalities were only a minor factor since it was found that  $\dot{V}_A/\dot{Q}$  relationships do not worsen in exercising horses - this is in contrast to the situation in man (Hammond *et al.*, 1986; Wagner, Gale, Moon, Torre-Bueno, Stolp and Saltzman, 1986).

The conclusions of Wagner *et al.* (1989) were supported by Anderson *et al.* (1989) who reported a widening end tidal-arterial  $P_{O_2}$  difference ( $P_{(ET-a)O_2}$ ) with increasing work rate in Thoroughbred horses, associated with a decrease in arterial-end tidal  $P_{CO_2}$  ( $P_{(a-ET)CO_2}$ ), suggesting improved uniformity of  $\dot{V}_A/\dot{Q}$  (Whipp and Wasserman, 1969). Arterial hypercapnia and a decrease in the ratio of alveolar ventilation to carbon dioxide production ( $\dot{V}_A/\dot{V}_{CO_2}$ ) were presented as evidence for hypoventilation at the highest exercise levels.

Bayly *et al.* (1989) described  $P_{aCO_2}$  changes at varying exercise intensities, finding hypocapnia during mild and moderate exercise, normocapnia in moderate to heavy exercise

and hypercapnia during very heavy exercise. In very heavy exercise of four minutes duration, hypoxaemia persisted whilst  $P_a\text{CO}_2$  gradually fell to below resting levels. They proposed that in short term strenuous exercise, horses hypoventilate, allowing  $P_a\text{CO}_2$  to rise and tolerating the resultant acidosis since to mount a hyperventilatory response sufficient to maintain normocapnia in the face of the horse's extremely high  $\dot{V}\text{CO}_2$  would be costly in terms of ventilatory work load. With more prolonged exercise however,  $\dot{V}_A$  is increased sufficiently to prevent  $\text{CO}_2$  retention (although insufficiently to resolve hypoxaemia and acidosis) thus indicating that the hypoventilation seen initially is not imposed by obligatory mechanical constraints.

Hodgson *et al.* (1990) reported that  $P_a\text{O}_2$  was lowest after two minutes of exercise at 62% and 100%  $\dot{V}\text{O}_{2\text{max}}$  and then returned to resting values at the lower work level but remained low at  $\dot{V}\text{O}_{2\text{max}}$ .  $P_a\text{CO}_2$  was unchanged at both work levels. Evidence was presented for an increase in  $\dot{V}_A$  during exercise without alteration in respiratory-locomotory coupling.

In contrast to the horse, ponies appear to maintain  $P_a\text{O}_2$  at resting levels during strenuous exercise (Bisgard *et al.*, 1978; Parks and Manohar, 1983; Parks and Manohar, 1984). Although Bisgard *et al.* (1978) found no increase in  $P_{(A-a)}\text{O}_2$  during low-speed ( $\leq 3.1 \text{ m}\cdot\text{s}^{-1}$ ) but strenuous (15% incline) work, Parks and Manohar (1984) believed it likely that during galloping ( $\approx 8.9 \text{ m}\cdot\text{s}^{-1}$ ) this variable was increased.

Bisgard *et al.* (1978) showed an increase in alveolar oxygen tension ( $P_A\text{O}_2$ ) during low-speed, strenuous exercise in ponies. Bayly *et al.* (1987a) found an increase in  $P_A\text{O}_2$  with the onset of exercise in Thoroughbreds but no further increase as work load increased, they

proposed that mechanical considerations may be the reason that it does not increase more.

To summarise, there has been a considerable amount of work investigating the respiratory response to exercise in the horse, its relationship to gait and its effect on gas exchange. Many of these studies have been performed using equipment which is either limited in its range of measurements or has a significant effect on breathing. There is still, therefore, room for considerably more work to characterise the nature of ventilation and gas exchange in the exercising horse.

### **(iii) The Effects of Training on Respiratory Function**

Horses have been prepared or 'trained' for use as sporting competitors or working animals for many centuries. The Hittite Kikkuli text from Anatolia (1,350 B.C.) contains instructions on the training of cavalry horses which bear elements of interval training, whilst the Athenian Xenophon (400 B.C.) also described the training of cavalry horses (McMiken, 1990).

The aims of training programmes have been summarised as delaying the onset of fatigue, improvement of speed and improvement in biomechanical skills (Rose, 1989). The relative importance of these aims varies according to the pursuit for which the horse is being prepared.

The results of individual investigations of the effects of training on physiological variables are liable to differences dependent upon the types of training regime used, their duration and possibly the previous training history of the animals used. In addition, variations in testing methods and the variables measured make comparisons of studies still more difficult.

Training-induced increases of  $\dot{V}O_{2\max}$  in horses have been reported. Evans and Rose (1988b) found a 23% increase in  $\dot{V}O_{2\max}$  following seven weeks of submaximal training in Thoroughbreds. Knight, Sinha and Rose (1991) reported an increase of around 10% after only two weeks of sub-maximal treadmill training, with no further increase over a further four weeks. In the latter study,  $\dot{V}O_{2\max}$  decreased towards pre-training levels after two weeks of detraining. Art and Lekeux (1993) reported an increase in peak  $\dot{V}O_2$  at levels at, or near to,  $\dot{V}O_{2\max}$  in Thoroughbred horses following three weeks light paddock exercise



followed by six weeks training.

Evans and Rose (1988b) found an increase in maximum cardiac output ( $\dot{Q}_{\max}$ ) and stroke volume (SV), a decrease in arteriovenous oxygen content difference ( $C_{(a-\bar{v})O_2}$ ) and no change in heart rate in association with the increase in  $\dot{V}O_{2\max}$  with training. They suggested that the main reason for the increase in  $\dot{V}O_{2\max}$  is the training-induced increase in  $\dot{Q}_{\max}$ . In contrast, Knight, Sinha and Rose (1991) reported an increase in  $C_{(a-\bar{v})O_2}$  following training,  $C_aO_2$  being significantly increased and  $C_{\bar{v}O_2}$  tending to be reduced. Bayly, Gabel and Barr (1983) found no significant change, but an upward trend in  $C_{(a-\bar{v})O_2}$ , whilst in the study of Thornton *et al.* (1983), no clear trend was revealed.

In sub-maximal exercise tests,  $\dot{V}O_2$  and  $\dot{Q}$  at a given exercise level did not appear to change with training (Thomas Fregin, Gerber and Ailes, 1983; Thornton *et al.*, 1987). Milne, Gabel, Muir and Skarda (1977) had earlier reported no change in immediate post-exercise  $\dot{Q}$  in Standardbreds pulling carts in a sub-maximal test. In contrast, Bayly, Gabel and Barr (1983) reported a decrease in sub-maximal  $\dot{Q}$  with training and Thornton *et al.* (1983) found variable effects.

Whilst Milne *et al.* (1977) found no significant change in post-exercise heart rate and SV during sub-maximal exercise, Bayly *et al.* (1983) found heart rate to be less at a given sub-maximal exercise level following training, whilst SV showed a non-significant trend to increase. Thomas *et al.* (1983), Pelletier *et al.* (1987) and Sexton, Erickson and Coffman (1987) found a decrease in heart rate with training in sub-maximal exercise tests, Thomas *et al.* also reporting an increase in SV. In ponies, Sexton, Argast and Erickson (1985) also

found a reduction in heart rate during exercise following training.

Milne *et al.* (1976) found a reduction in post-exercise  $P_{\bar{v}O_2}$  following training, which they suggested may represent an increased oxygen extraction by the working muscles. Thornton *et al.* (1983) found no effect of training on arterial or mixed venous blood gases or pH during exercise. In contrast, Sexton, Erickson and Coffman (1987) found that training prevented the decrease in  $P_aO_2$  during low-speed ( $\leq 2.8 \text{ m}\cdot\text{s}^{-1}$ ) exercise seen in untrained Standardbreds, although the same group found a *decrease* in  $P_aO_2$  at similar exercise levels following training in ponies (Sexton, Argast and Erickson, 1985). When the Standardbreds trained by Sexton, Erickson and Coffman (1987) were subsequently detrained, Erickson, Sexton, Erickson and Coffman (1987) reported in their paper's summary no effect of detraining on arterial blood gases and pH, however no data for this were shown in the text.

Sexton, Erickson and Coffman (1987) reported an increase in  $P_aCO_2$  at rest and all levels of exercise in Standardbreds following training, but an unchanged response of this variable to exercise, i.e. a reduction with increasing speed. In contrast in ponies, Sexton, Argast and Erickson (1985) found no change in resting or exercising  $P_aCO_2$  with training.

Arterial pH ( $pH_a$ ) at rest and during exercise was reported to remain unchanged by training (Sexton, Argast and Erickson, 1985) and detraining (Erickson *et al.*, 1987, again, reported only in the summary to this paper) in Standardbreds, but was increased during exercise in ponies following training (Sexton, Argast and Erickson, 1985).

An increase in resting and exercising blood haemoglobin concentration with training has

been reported by numerous authors (Milne *et al.*, 1976; Allen and Powell, 1983; Knight, Sinha and Rose, 1991). Although Sexton, Erickson and Coffman (1987) and Erickson *et al.* (1987) found no significant change in resting or exercising haemoglobin concentration in consecutive studies of training, detraining and retraining in Standardbred horses, they recorded the highest exercise haemoglobin concentrations in their final test, i.e. at the end of the second period of training.

Although there have been numerous studies of the cardiovascular response to training and its effect on oxygen delivery, there are relatively few reports concerning the ventilatory response to training. Pelletier *et al.* (1987) studied the effect of training on the ventilatory response to exercise using seven Standardbreds and one Quarterhorse. Both training and exercise testing were carried out at relatively low speeds and had little effect on minute ventilation ( $\dot{V}_e$ ),  $V_t$ ,  $f_b$ , physiological dead space ( $V_{dphys}$ ),  $V_{dphys}/V_t$  or  $\dot{V}_A$ . Evans and Rose (1988b) found that no increase in  $\dot{V}_e$  occurred with the 23% increase that they reported in  $\dot{V}_{O_{2max}}$ .

Recently Art and Lekeux (1993) reported that  $\dot{V}_e$ ,  $f_b$  and  $V_t$  were unaltered by training although peak  $\dot{V}_{O_2}$  increased significantly. They suggested that the decrease in ventilatory equivalent ( $\dot{V}_e/\dot{V}_{O_2}$ ) must be associated with improved alveolar oxygen extraction.

A reduction in exercising  $t_{pa}$  and  $t_{re}$  in horses with training has been reported respectively by Sexton, Erickson and Coffman (1987) and by Bayly, Gabel and Barr (1983). A similar response to training was reported in ponies by Sexton, Argast and Erickson (1985).

Numerous authors have reported that training of horses and ponies results in a reduction in blood lactate accumulation during strenuous exercise (Milne *et al.*, 1976; Persson, Essen-Gustavsson, Lindholm, McMiken and Thornton, 1983; Thornton *et al.*, 1983; Sexton, Argast and Erickson, 1985; Sexton, Erickson and Coffman, 1987; Sloet van Oldruitenborgh-Oosterbaan, 1990) and this parameter has been used as an index to monitor the effects of training.

Detraining of Quarterhorses (by six months of paddock rest) was reported to have no significant effect on various cardiovascular parameters during low speed exercise ( $2.8 \text{ m.s}^{-1}$  on a  $7^\circ$  incline), including heart rate. Arterial blood gases, pH, haemoglobin and lactate during exercise showed no significant changes, although lactate and peak  $t_{\bar{v}}$  showed a tendency to rise (Erickson *et al.*, 1987). The horses appeared to find the exercise test more difficult to complete when detrained and they seemed to sweat more profusely.

Butler *et al.* (1991) found no significant effect of cessation of training (for fifteen weeks) of Thoroughbred horses on  $\dot{V}O_{2\text{max}}$ . At  $10\text{-}11 \text{ m.s}^{-1}$ ,  $\dot{V}CO_2$ , inspiratory tidal volume ( $V_i$ ), heart rate, SV,  $\dot{Q}$ , haemoglobin concentration [Hb],  $P_aO_2$ ,  $P_aCO_2$ ,  $pH_a$ , arterial and mixed venous oxygen contents ( $C_aO_2$  and  $C_{\bar{v}}O_2$ ) and arterial temperature were unchanged. The only measured variable to alter was blood lactate concentration which more than doubled after fifteen weeks out of training. In contrast, Knight, Sinha and Rose (1991) found a decrease in  $\dot{V}O_{2\text{max}}$  with as little as two weeks of detraining and Art and Lekeux (1993) found a decline in  $\dot{V}O_{2\text{max}}$  following three weeks of detraining.

The relative scarcity of studies describing the ventilatory response to training in the horse, coupled with the conflicts in reported changes in cardiovascular and blood gas responses indicates the need for further studies of the effects of training in this species.

#### **(iv) Equine influenza**

The detection of antibody production against an influenza A myxovirus in the horse was first reported in 1955 (Heller, Espmark and Viriden, 1956) in animals convalescing from respiratory disease. Epizootics of this condition occurred in Sweden in 1955 and 1956. A similar disease occurred in Dresden in 1956 and spread extensively over Eastern Europe (Doll, 1963). The agent responsible was isolated in May, 1956 from horses in Prague and identified as a subtype of *Myxovirus influenza A* (Sovinova *et al.*, 1958). The virus was designated A/equi/Prague/56 (Sovinova *et al.*, 1958).

Serological surveys suggested that horses in a number of European countries had encountered influenza (Kaplan and Payne, 1959), whilst in the United States, Doll (1961) demonstrated haemagglutination inhibition (HI) antibody against A/equi/Prague/56 virus in a large proportion of brood mares and yearlings, unassociated with clinical disease suggesting that this or a related virus had infected horses in the USA since at least 1957.

A highly infectious outbreak of respiratory disease in Florida in February 1962 affecting mostly horses of three or over with a 60 to 70% morbidity in this age group, was described by Waddell *et al.* (1963). Very young and relatively old horses were worst affected. These workers showed the disease to be due to an influenza virus which was antigenically distinct from those previously reported. The new strain became known as A/equi-2/Miami/1/63 and was the cause of epizootic respiratory disease throughout the USA and south-central Canada in 1963, spreading the following year to Brazil, Uruguay and Argentina and to Europe in 1965 (Bryans, Doll, Wilson and Zent, 1967).

In April 1963, there was a widespread outbreak of respiratory disease in racehorses in Kentucky which was shown, by virus isolation, to be due to an agent closely related to the Prague strain, designated A/equine/1/Lexington/1/63 (Bryans, Doll, Wilson and Zent, 1967). In this outbreak signs of disease were mostly confined to horses aged two years old or less. Pre-infection HI titres from affected animals were lower than titres of unaffected horses and seroconversions were seen only in animals with pre-infection titres of 1:20 or less. It appeared that horses aged three or more had a protective immunity probably obtained due to intermingling of the racing population in the USA, resulting in increased exposure to viral challenge (Bryans *et al.*, 1967). In contrast, an A/equine/1 epizootic of respiratory disease occurred in Mexico in 1964 in a relatively closed horse population with low pre-infection HI titres affecting 75% of horses with no age-related immunity being seen (Bryans *et al.*, 1967).

It has already been mentioned that respiratory disease described as 'equine influenza', clinically similar to the disease observed in outbreaks of confirmed equine influenza, have occurred for many years in Great Britain. In relatively recent times, epizootics of disease sufficiently widespread to interfere with the racing programme occurred in 1935, 1947, 1955 and 1959 (Beveridge *et al.*, 1965), with less severe outbreaks in between. It was not until 1962 however, that serological evidence of exposure to influenza A/equine/1 was described in the British Isles in a small percentage of horses in Northern Ireland (Meenan, Boyd and Mullaney, 1962). A severe outbreak of respiratory disease which occurred in horses in England in 1963 was shown by Beveridge *et al.* (1965) to be due to an A/equine/1 virus which became designated A/equine/1/Cambridge/63. By retrospective serological analysis, these workers were able to demonstrate that A/equine/1 infection had

occurred in horses in England at least as far back as 1948, but that influenza A/equine/2 had probably not occurred in this country (Beveridge *et al.*, 1965).

A/equi-2 influenza reached Great Britain in March 1965, initially causing disease in stud farms in England and Ireland, but soon spreading throughout the Thoroughbred and non-Thoroughbred population (Beveridge, 1965). Most stables were affected except those in which vaccination had been carried out earlier in the year (Miller, 1965 and 1966). In young foals especially, secondary bacterial infections sometimes resulting in a fatal pneumonia occurred (Miller, 1965 and 1966). It was found that prior infection with A/equine/1 strains of influenza conferred no protective immunity against the A/equine/2 strain (Miller, 1966).

Influenza caused by A/equine/2 virus was again widespread in Great Britain in 1969 (Rose, Round and Beveridge, 1969). In horses the clinical picture was similar to previous outbreaks, with high morbidity but almost zero mortality, although significant numbers of deaths were reported in donkeys.

New influenza strains tend to replace previously-occurring ones in a given species (Powell, Thomson, Spooner, Plowright, Burrows and Schild, 1974), so that although isolated outbreaks of disease were reported in 1968 and 1972, a widespread epidemic in 1973 due to the A/equine/1 strain was unexpected (Powell *et al.*, 1974). Although slight antigenic differences were found between the new strain and previous sub-types, horses vaccinated with preparations containing the prototype strain did not develop signs of disease (Bowen, 1973; Powell *et al.*, 1974).



Throughout the nineteen seventies, influenza continued to affect the British horse population. Unvaccinated animals were affected by localised outbreaks due to A/equine/2 in 1971 and 1972 and due to A/equine/1 in 1977 (Mumford and Rossdale, 1980; Baker, 1986). In 1976, an outbreak of disease due to an A/equine/2 strain occurred in which for the first time it was reported that vaccinated horses were affected, albeit to a lesser degree than unvaccinated horses (Thomson, 1977). This outbreak, for the most part restricted to the Newmarket area, was thought to have been caused by the importation of an infected animal from the USA.

Between December 1978 and June 1980 an enzootic due to an A/equine/2 strain, designated A/equine/2/England/1/79 occurred in Great Britain (Burrows, Goodridge, Denyer, Hutchings and Frank, 1982). Initially affecting unvaccinated and inadequately vaccinated horses, it later caused disease in vaccinated animals. In some stables mixed infections involving influenza and other respiratory viruses (equine herpes virus-1 and rhinovirus 2) were detected. Studies of affected, vaccinated horses in one stable suggested that whilst antibody titres in these animals were adequate for protection against an A/equine/1 challenge, they were insufficient to prevent A/equine/2 infection (Burrows *et al.*, 1982). Although the mortality in this episode was again extremely low, it was reported that some affected racehorses took several months to recover fully from the effects of infection and that some horses never recovered their previous racing ability (Livesay, 1991).

Widespread outbreaks of influenza also occurred in Europe (Klingeborn, Rockborn and Dinter, 1980; van Oirschot, Masurel, Huffels and Anker, 1981) and the USA (Hinshaw,

Naeve, Webster, Douglas, Skehel and Bryans, 1983) at around this time and virus isolates from these outbreaks were shown to be variants of the reference A/equine/2/Miami/63 strain (Hinshaw *et al.*, 1983). Following these outbreaks vaccine manufacturers began to incorporate a representative of the variant strains into vaccines (Baker, 1986).

Great Britain remained free of influenza for almost ten years after this epidemic with the exception of minor episodes in 1980, 1981 and 1984, when animals were imported to the country whilst incubating the disease (Livesay, 1991). In 1989, however, a widespread outbreak of influenza occurred due to an A/equine/2 subtype. The infection spread rapidly during the summer months and both vaccinated and unvaccinated horses were affected, although signs in vaccinated animals were far less severe (Livesay, Yadav, O'Neill and Mumford, 1992). The agent involved (A/equine/2/Suffolk/89), showed evidence of genetic and antigenic drift, which it has been suggested may have contributed to the failure of vaccines to control the disease (Mumford, 1992). In July 1991 the infection reappeared involving the same virus subtype and was again widespread amongst the equine population, although infection was mainly confined to two and three year olds, the older horses presumably retaining some residual immunity from the previous outbreak (Livesay, 1991).

Currently equine influenza is an important respiratory viral pathogen which, since its introduction to South Africa in 1986 and India in 1987 (Mumford, 1992), now affects all areas of the world with the exception of Australia and New Zealand. In 1989, a severe influenza epizootic occurred in China where morbidity in some areas was 80% and mortality in the fully susceptible population 20% (Mumford, 1992). The cause was an A/equine/2 strain which has been shown to be antigenically considerably different from

other strains in this group (Guo, Wang and Zheng, 1990). It has been suggested that this strain is of avian origin, but no longer able to affect its original avian host (Webster and Guo, 1991).

It appears that the A/equine/2 subtype is now the dominant pathogen as the last isolation of subtype 1 was made in Mongolia in 1980, since when there has been only serological evidence of its existence in Asia and Central Europe (Anon. 1987).

In view of the close relationship between the equine influenza virus and the influenza A viruses of man, there has been some concern as to whether equine influenza may be transmissible to man. Such speculation is not new, indeed as early as the seventeenth century it was suggested that influenza in horses may be transmissible to man (Molineux, 1695). More recently it has been suggested that there is an antigenic relationship between A/Equi-2 influenza virus and the 1968 Hong Kong variant of human type A influenza virus (Coleman, Dowdle, Pereira, Schild and Chang, 1968). However, although in the nineteen sixties it was shown that ponies could be experimentally infected with human influenza B virus (Kasel, Byrne, Harvey and Shillinger, 1968) and human volunteers were susceptible to experimental inoculation of A/Equine/2/Miami/1/68 virus (Cough, Douglas, Kasel, Riggs and Knight, 1969), it appears unlikely that such cross-infections play a part in the normal natural history of these viruses. In the 1989 influenza outbreak attempts to isolate virus from nasal washes taken from grooms in charge of affected animals proved unsuccessful (Hannant, D., personal communication).

### *Influenza vaccination*

Inactivated vaccines against both sub-types of influenza became available in Europe and North America in the early nineteen sixties (Bryans, Doll, Wilson and McCollum, 1966). As detailed above, vaccination has been successful in reducing the effects of epizootics of equine influenza although it does not provide complete protection. Mumford, Wood, Scott, Folkers and Schild (1983), Wood, Schild, Folkers, Mumford and Newman (1983a) and Wood, Mumford, Folkers, Scott and Schild (1983b) demonstrated that the single radial haemolysis technique (SRH) allows the estimation of levels of antibody associated with immunity and the assessment of the probable duration of immunity provided by vaccines of known antigen content, as well as demonstrating the beneficial effect of adjuvants in inactivated virus vaccines.

The frequency at which booster vaccinations should be carried out has been discussed by various authors. It appears that the more booster injections a horse receives, the longer is the duration of the antibody response (Ingram, Sherman, Mitchell, Thorsen, Martin and Barnum, 1978; WHO, 1983), although too short a vaccination interval has no beneficial effect and may actually depress antibody levels (WHO, 1983). For these reasons Baker (1986) recommended that young animals should be boosted more frequently than older ones, i.e. at four to six month intervals, but that more frequent vaccination may be contra-indicated. A vaccination interval of six months rather than twelve was also recommended by Plateau, Jacquet and Cheyroux (1988).

By 1970 the use of influenza vaccines in horses in training in Britain was fairly widespread (Mumford and Rossdale, 1980) but it was not until 1980 that vaccination of all

Thoroughbred horses in training became compulsory under Jockey Club rules. This policy was soon adopted by many other breed societies and competitive organisations. It has been suggested that this policy was at least in part responsible for the ten year gap which occurred before the outbreak of equine influenza in Great Britain in 1989, although Baker (1986) pointed out that considerably less than 25% of the British equine population was vaccinated and it has been suggested that a vaccination rate of over 70% is necessary to prevent influenza epidemics.

Despite the reduced incidence and severity of influenza infections associated with vaccination, within the racing industry there is some opposition to the mandatory vaccination policy by some owners and trainers who report adverse effects of vaccination. A survey by Livesay showed that amongst racehorse trainers vaccination was considered a common cause of adverse reactions, the commonest being loss of performance, with or without overt signs of respiratory disease (Livesay, G., personal communication). However, whilst suspected systemic reactions to vaccination have been reported (Eagles and Higgins, 1985; Clarke, 1985; Matthews, 1986; Mair, 1986) their incidence is low and Baker (1986) showed that the actual reported incidence of vaccine reactions was only 0.011%, with local reactions predominating, the systemic reaction rate being only 0.006%.

In view of the incomplete immunity conferred by vaccination, there has been much interest in the development of more efficacious preparations. In the 1990s, new vaccines have become available, one based on immune stimulating complexes (ISCOMS) as an antigen presentation method (*Equip*, Mallenkrodt Ltd., Crewe) the others representing improvements to existing vaccines by the addition of new adjuvants (*Duvaxyn IE Plus*,

Solvay Duphar Veterinary, Southampton; *Prevac Plus*, Hoechst U.K. Ltd., Milton Keynes). Whilst all vaccines currently in use for influenza vaccination in the horse are inactivated preparations, various forms of live, attenuated vaccine are under investigation including temperature sensitive reassortments (Brundage-Anguish, Holmes, Hosier, Murphy, Massicott, Appleyard and Coggins, 1982; Holmes, Lamb, Anguish, Coggins and Gillespie, 1988; Holmes, Lamb, Coggins, Murphy and Gillespie, 1992), Vaccinia recombinants (Dale, Brown, Kloss, Cordell, Moore and Yilma, 1988) and avian/equine reassortments (Mumford, 1992).

### *Experimental studies of equine influenza*

Although transmission and immunisation studies on equine influenza were reported as long ago as the nineteen forties (Jones, Gleiser, Maurer, Hale and Roby, 1948), such work was carried out at a time when no differentiation had been made between influenza and other equine pathogens such as equine herpesvirus and equine arteritis virus. Indeed the studies by Jones *et al.* would appear more likely to have involved EHV rather than influenza as transmission from viraemic animals was possible. Since the identification of equine influenza as a definite viral entity (Heller *et al.*, 1956; Sovinova *et al.*, 1958), many studies have been carried out into clinical, pathological and immunological aspects of the disease, involving both case material from naturally-occurring outbreaks of disease and from experimental challenge studies.

Bryans, Doll, Wilson and McCollum (1966) described the use of the first equine influenza vaccine and the humoral antibody response to it. They found that HI titres of 1:40 or greater were associated with protection from the disease and suggested that the antigenicity

of A/equi-1 strains was greater than that of A/equi-2, in terms of stimulation of antibody response. Other workers reported levels of antibody consistent with immunity from infection ranging from 1:8 to 1:128 (Rouse and Ditchfield, 1970b; Bryans, 1973; Kumanomido and Akiyama, 1975; Powell, Burrows, Spooner, Mumford and Thomson, 1977). Mumford *et al.* (1983) suggested that this disparity in results is partly related to variability in the sensitivity of the HI test, especially as applied to A/equi-2 influenza strains.

Rouse and Ditchfield (1970a, 1970b) demonstrated the production of local nasal antibody to influenza in ponies and an association between serum and nasal antibody levels. It appears likely that local antibody plays an important role in resistance to infection (Rouse and Ditchfield, 1970b; Kumanomido and Akiyama, 1975).

The HI test suffers from its inherent variability which means that a fourfold increase in antibody is necessary for diagnosis of infection. As experimental and field studies have shown that infection with virus shedding can occur in the absence of a fourfold rise in HI titres, the test may sometimes fail to detect infections (Mumford, 1990). For this reason, the single radial haemolysis (SRH) method of haemagglutinin assay (Schild, Pereira and Chakraverty, 1975) has been applied in the diagnosis of equine influenza (Bockman, 1977; Hamilton, 1978; Wood *et al.*, 1983b). The technique has been well standardised and is sensitive and reproducible (Plateau and Cruciere, 1983). Mumford *et al.* (1983) showed that SRH levels correlated well with the degree of clinical protection versus influenza challenge and that high levels were required for complete protection, SRH zones  $>65\text{mm}^2$  being necessary to protect against intranasal challenge.

Although early attempts at experimental challenge were unsuccessful in achieving infection or causing clinical signs (Doll, 1961; Lief and Cohen, 1965) unless subjects were stressed before infection, (Blaskovic, Szanto, Kapitancik, Lesso, Lackovic and Skarda, 1966), such challenges have become a standard method of investigation of the disease. Mumford, Hannant and Jessett (1990) examined the use of intranasal inoculation versus aerosol inhalation as methods of challenge. They found that the severity of signs and kinetics of virus shedding were dose related and concluded that aerosol inhalation was the more reliable method and is more suitable for experimental challenge studies. Much higher levels of SRH antibody, of the order of 120-154mm<sup>2</sup> were found to be necessary for protection against aerosol challenge (Mumford, 1992).

Duration of protection from influenza infection following vaccination has been disappointing, Wood *et al.* (1983b) reporting that adequate antibody titres for protection lasted for as little as four to six weeks following two doses of an aqueous vaccine and around twelve weeks following the use of an adjuvanted vaccine.

Hannant, Mumford and Jessett (1988a) investigated the duration of circulating antibody and immunity following influenza infection and found that in contrast to the vaccinated animal in which immunity is related to circulating antibody levels and is short-lived, in rechallenged ponies there was complete clinical protection for at least 32 weeks and partial protection for more than a year. Interestingly, this immunity persisted after circulating antibody levels had waned. These authors concluded that 'immune mechanisms which operate after infection with influenza differ in some respects from those which confer vaccine-induced protection from challenge' and drew attention to the probable involvement



of local antibody and cell mediated immunity (CMI) in resistance to reinfection, as is the case in man (Callard, 1979).

Further studies have investigated antibody isotype responses, both systemic and local, to infection and vaccination and showed a local anamnestic response to rechallenge (Hannant, Jessett, O'Neill, Sundqvist and Mumford, 1988b; Hannant, Jessett, O'Neill and Mumford, 1989), whilst early studies of CMI have demonstrated the presence of cytotoxic lymphocytes in peripheral blood following infection of ponies (Hannant *et al.*, 1988b; Hannant and Mumford, 1989).

The effects of influenza infection on tracheal mucus clearance rates were investigated by Willoughby, Ecker, McKee, Ridolls, Vernaillen, Duboui, Lein, Mahony, Chernesky and Magy (1991) who showed a significant reduction in clearance rates for four to five weeks following infection of naive ponies.

Despite the now considerable amount of research which has been carried out into equine influenza, nearly all challenge studies appear to have been confined to ponies. In view of the substantial differences between ponies and Thoroughbreds, there is a need for challenge studies to investigate the effects of influenza on immunity and respiratory function in the latter breed. Similarly, although the clinical response to infection, immunology and pathology of equine influenza have been investigated, the effects of the disease on respiratory function do not appear to have received attention.

## CHAPTER THREE - MATERIALS AND METHODS

### **(i) Horses**

All studies were carried out on Thoroughbred horses. The sex and ages of the experimental animals involved are shown in Table 3.1. They were housed in loose boxes, bedded on shredded paper and fed a diet of proprietary cubed feed (Racehorse Nuts; Dodson and Horrell, Ringstead) and hay. When in full work they were fed approximately eight kilograms of cubes and seven kilograms of hay per day, with amounts being reduced during periods of rest or reduced work. All horses were fully acclimatised to treadmill exercise and endoscopy prior to being used in the studies.

### **(ii) Standardised Exercise Tests**

#### *Exercise Regime*

Standardised Exercise Tests (SETs) were carried out on a high speed treadmill (Sato, Sweden - Plate 3.1), housed in an air conditioned laboratory. Tests were performed with the ambient temperature kept as near as possible to 20°C and relative humidity maintained as close to 60% as possible.

During exercise, the horses wore a padded leather surcingle around the chest, loosely attached to a safety strap of sufficient strength to support the horse's weight in case of a fall. The strap was connected to a load-activated trip switch on the treadmill A-frame so that in the event of an accident, the horse's weight would operate the switch and turn off the treadmill. A domestic electric fan was placed in front of the horse at chest level in order to provide airflow and assist in cooling the animal. During exercise, the horse was

controlled by means of a snaffle bridle, the reins being held by two grooms standing one on either side of the horse.

Following an initial warm up period, consisting of ten minutes walk ( $1.6 \text{ m.s}^{-1}$ ), three minutes trot ( $3.2 \text{ m.s}^{-1}$ ) and five minutes walk ( $1.6 \text{ m.s}^{-1}$ ), an interrupted, multistep exercise regime was used in which two minute bouts of canter were separated by ten minute periods of recovery at walk ( $1.6 \text{ m.s}^{-1}$ ). The regime is summarised in Figure 3.1.

The canter speeds used in each test are given with the description of each study, but varied in intensity from six  $\text{m.s}^{-1}$  on a level surface to twelve  $\text{m.s}^{-1}$  on a three degree incline.

### **(iii) Preparation of the Horse**

Immediately prior to testing, horses were weighed and then stood in a set of stocks in the exercise unit to facilitate preparation, which was carried out as follows.

Small areas of skin over a transverse facial artery and the left jugular vein were shaved and aseptically prepared using povidone-iodine (Povidine Surgical Scrub; BK Veterinary Products, Bury St. Edmunds) and surgical spirit (J.M. Loveridge, Southampton). Following local anaesthesia of the skin with 2% lignocaine hydrochloride, a 20G sampling catheter

**Table 3.1: Age and sex of experimental horses used in the studies**

<i>Horse</i>	<i>Age</i>	<i>Sex</i>	<i>Studies</i>
Briny	8	gelding	R, K/N, Tr, V3
Buddy	10	gelding	K/N, Tr, V3
Flighty	7	mare	R, K/N, Tr, V3
Goldie	7	mare	R, K/N, Tr, V3
Monty	4/5	gelding	K/N, Tr, V2, V3
Ross	7	gelding	K/N, Tr, V3
Hero	9	gelding	V1
Killer	11	gelding	V1
Merry Ridge	9	gelding	V1, V2
Spock	4	gelding	V2
Cooper	6	gelding	R

R

=

right atrial sampling site validation  
(Chapter 5)

K/N

=

kinetics of respiratory response and normal  
response studies (Ch.6 & 7)

Tr

=

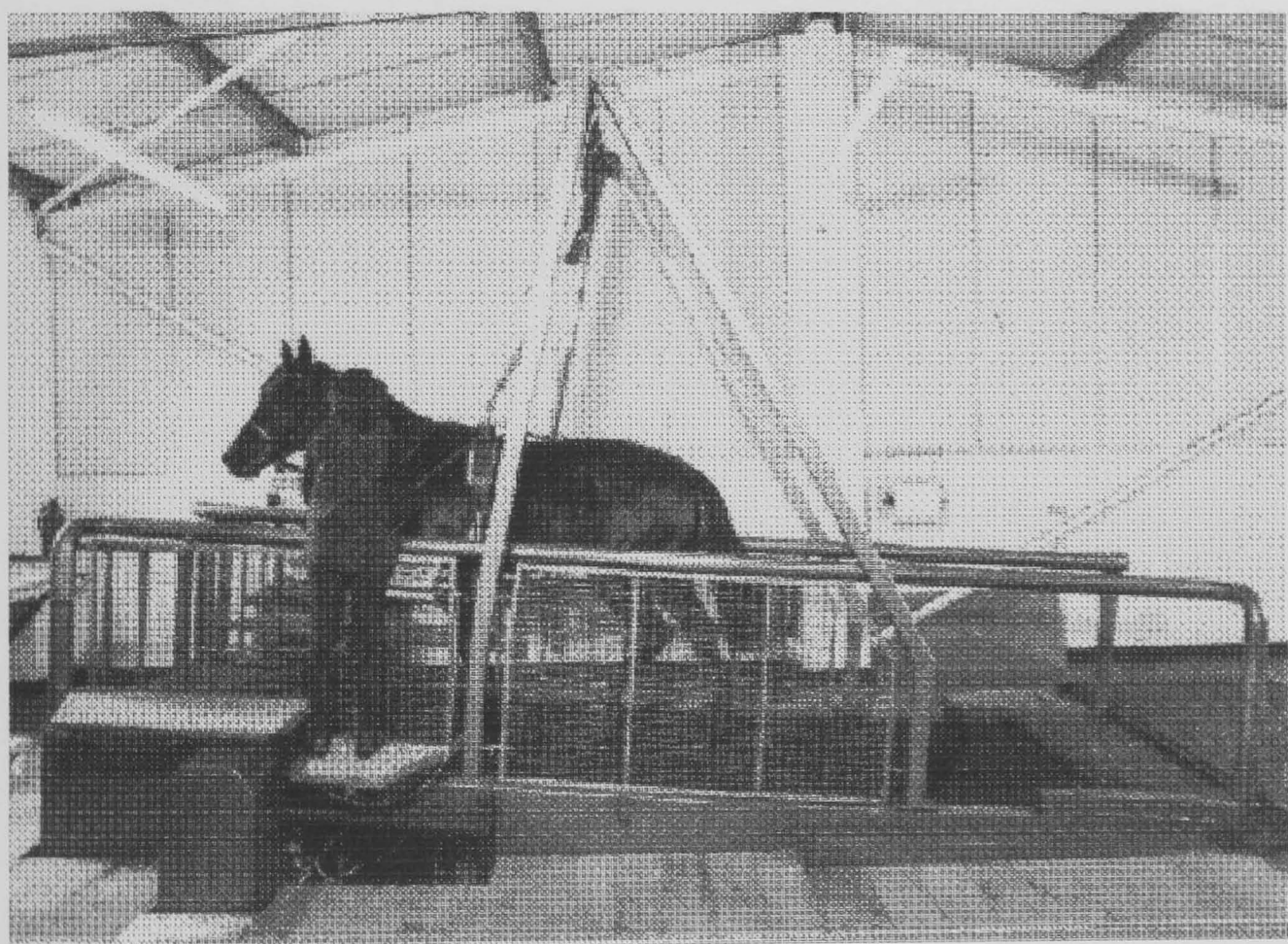
training study (Ch. 8)

V1-3

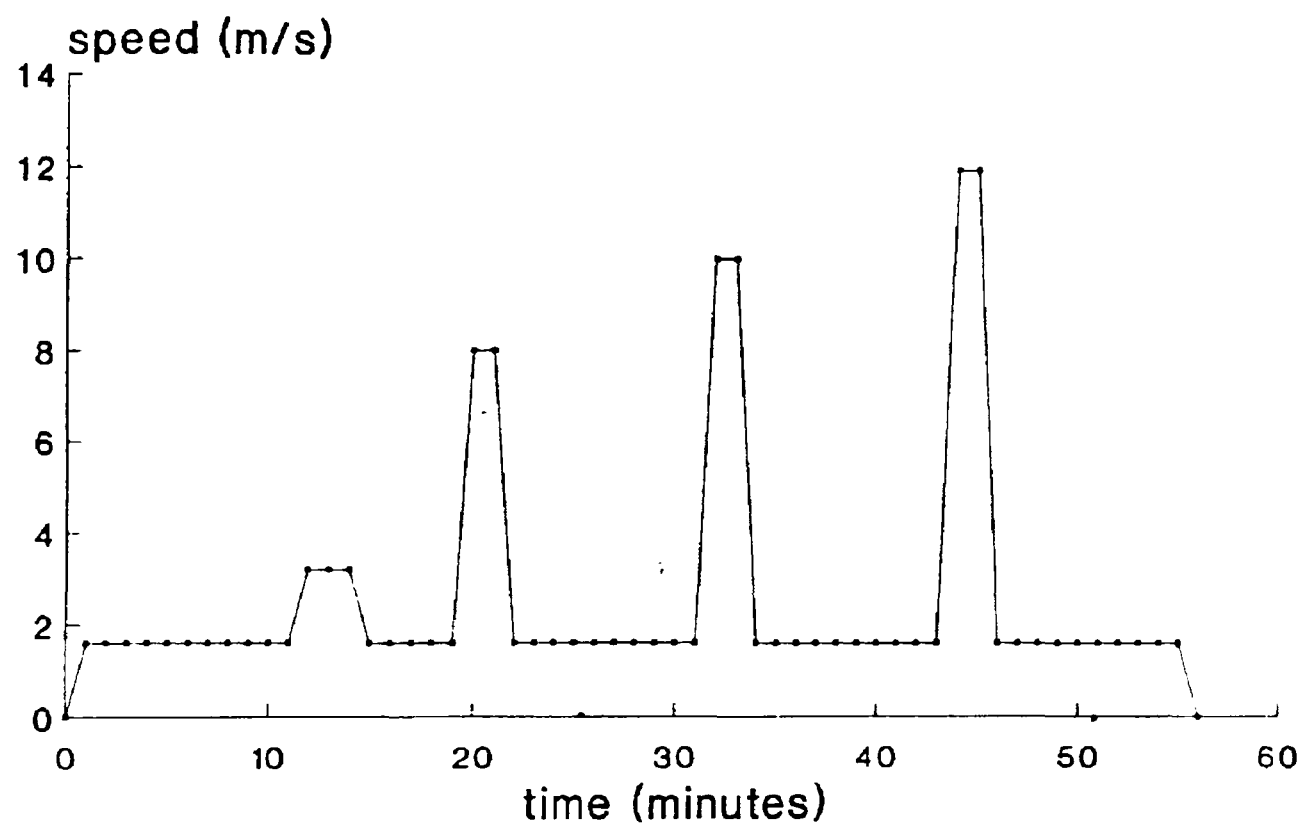
=

viral challenges one to three (Ch. 9 & 10).

**Plate 3.1: The Sato High Speed Treadmill**



**Figure 3.1: Exercise test protocol**



(Critikon, Tampa, USA) was placed in the transverse facial artery, fixed in place with superglue (Super Glue 4; Bostik, Leicester) and connected to a two metre extension tube with a dead space of 1.7ml (lectro-cath; Vygon, Ecouen, France) to permit arterial blood sampling during exercise.

Following similar local anaesthesia, an 8F diameter percutaneous catheter introducer (Arrow International, Philadelphia, USA) was located in the left jugular vein and fixed in place with a single skin suture. A 7F thermodilution catheter (Viggo, Swindon) was inserted via the introducer, passed along the jugular vein and located in the right atrium. A one metre extension tube with a dead space of 1.3ml (lectro-cath; Vygon, Ecouen, France) was connected to the distal sampling port of the catheter. Mixed venous blood samples were drawn through this port and mixed venous blood temperatures measured by means of the catheter's thermistor, the latter having been previously calibrated in water at 37°C and 41°C in a thermostatically-controlled waterbath (Tectron S-543; Lab-Plant, Huddersfield).

Both sampling lines were filled with heparinised saline (10 iu heparin/ml - Monoparin 5000 units/ml; CP Pharmaceuticals, Wrexham and Aquapharm 1; Animalcare) to maintain patency.

A lightweight, fibreglass facemask, padded to prevent leaks, was fitted over the horse's nostrils, secured in place by attaching it to the browband and cheekpieces of the bridle and held firmly over the muzzle by a chinstrap (Plate 3.2). As the horse is an obligate nasal breather, it was unnecessary for the mask to cover the mouth. This allowed the use of a

small mask which minimises apparatus dead space and permits the use of a conventional bridle and bit for control of the horse.

In an earlier study, using this system; (Butler *et al.*, 1993a) the mask was tested for leakage by adding an undertray to enclose the mouth and jaw and sealing this whole apparatus to the head using flexible plastic film. A third flow tube was fitted to the undertray to measure gas leaking from the facemask. When horses were exercised using this arrangement, peak flow rates from the undertray were  $\approx 3.6\%$  of peak flow through the mask. However, the flow profiles were dissimilar to those recorded at the nostrils and appeared related to head movement within the additional apparatus. Thus it was concluded that leakage of airflow around the mask was not an important source of error.

A circular opening in the mask over each nostril allowed the attachment of a plastic tube (length 10cm, functional internal diameter<sup>1</sup> 4.9cm) housing a pair of ultrasonic transducers for measurement of gas flow rates (Plate 3.2 and Figure 3.2). The inlet capillary (length 2m, internal diameter 0.38mm) of a rapid response quadrupole mass spectrometer (Airspec MGA 2000; Chest Scientific Instruments, Westerham) was positioned in the path of gas flow from the right nostril via a small hole in the flow transducer tube.

Heart rates were recorded using a commercial telemetric recording system (Hippocard PEH 200; Isler Bioengineering, Zurich) via skin electrodes, held in place by the girth, over the

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<sup>1</sup>An absorbent cloth lining was fitted into each flow tube, held in place with a tight fitting wire mesh liner, to absorb water vapour condensing in the flow tubes. The functional internal diameter refers to the lumen of the tube with these in place.



lateral and ventral thorax.

#### **(iv) Measurement of Respiratory Flow Rates and Gas Concentrations**

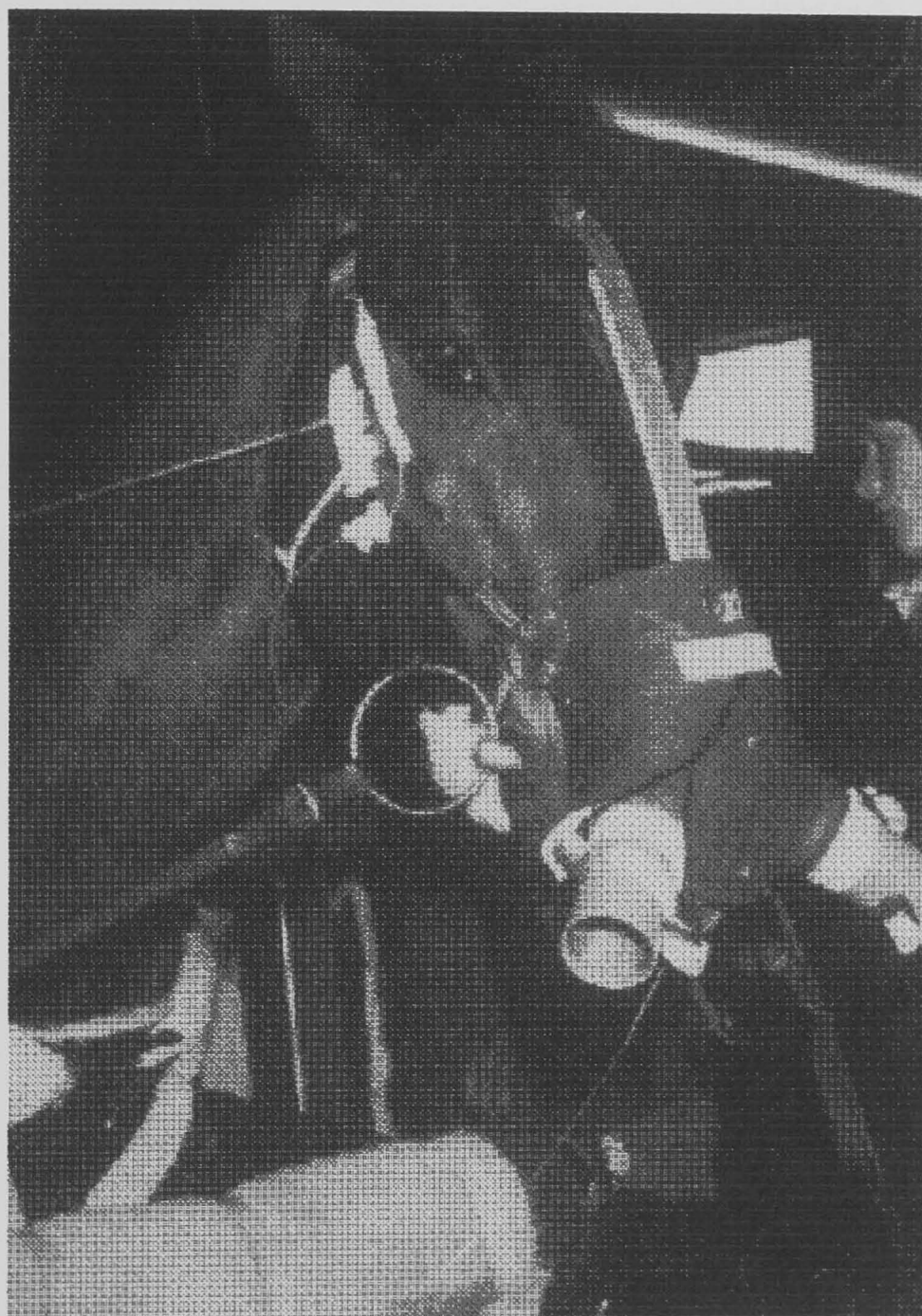
Respiratory flow rates were measured using ultrasonic flow transducers as described by Woakes, Butler and Snow (1987). Two transducers were mounted diagonally across each flow tube located in sealed plastic mounts so as not to interfere with gas flow (Figure 3.2). Windows in the tubes over each transducer provided an acoustic path between them. A 40KHz ultrasound signal was pulsed alternately back and forth between the transducers.

Gas flow causes a change in phase of the received signal which is linearly related to the velocity of the gas (Woakes and Butler, 1980). The output from each pair of transducers was passed through a processor which converted phase shift into an analogue signal corresponding to  $0.05 \text{ v/l.s}^{-1}$  gas flow. The system is now commercially available (Birmingham Research and Development, Birmingham).

The flow tubes were individually calibrated before each test using a fixed flow rate measured with a rotameter (KDG Flowmeters, Burgess Hill).

Respiratory gas concentrations were measured using the mass spectrometer. Air was continuously drawn through the inlet capillary at  $4 \text{ ml. min}^{-1}$  into the mass spectrometer for analysis. The sampled gas is ionised in a vacuum and subjected to the electric field of a quadrupole electrode. Within this field, ions of different mass/energy ratios are deflected to differing degrees. By varying the voltage applied to the electrode, gases of differing

**Plate 3.2: Facemask fitted to bridle. The flow tubes are visible over each nostril and the mass spectrometer capillary can be seen exiting the right hand flow tube.**



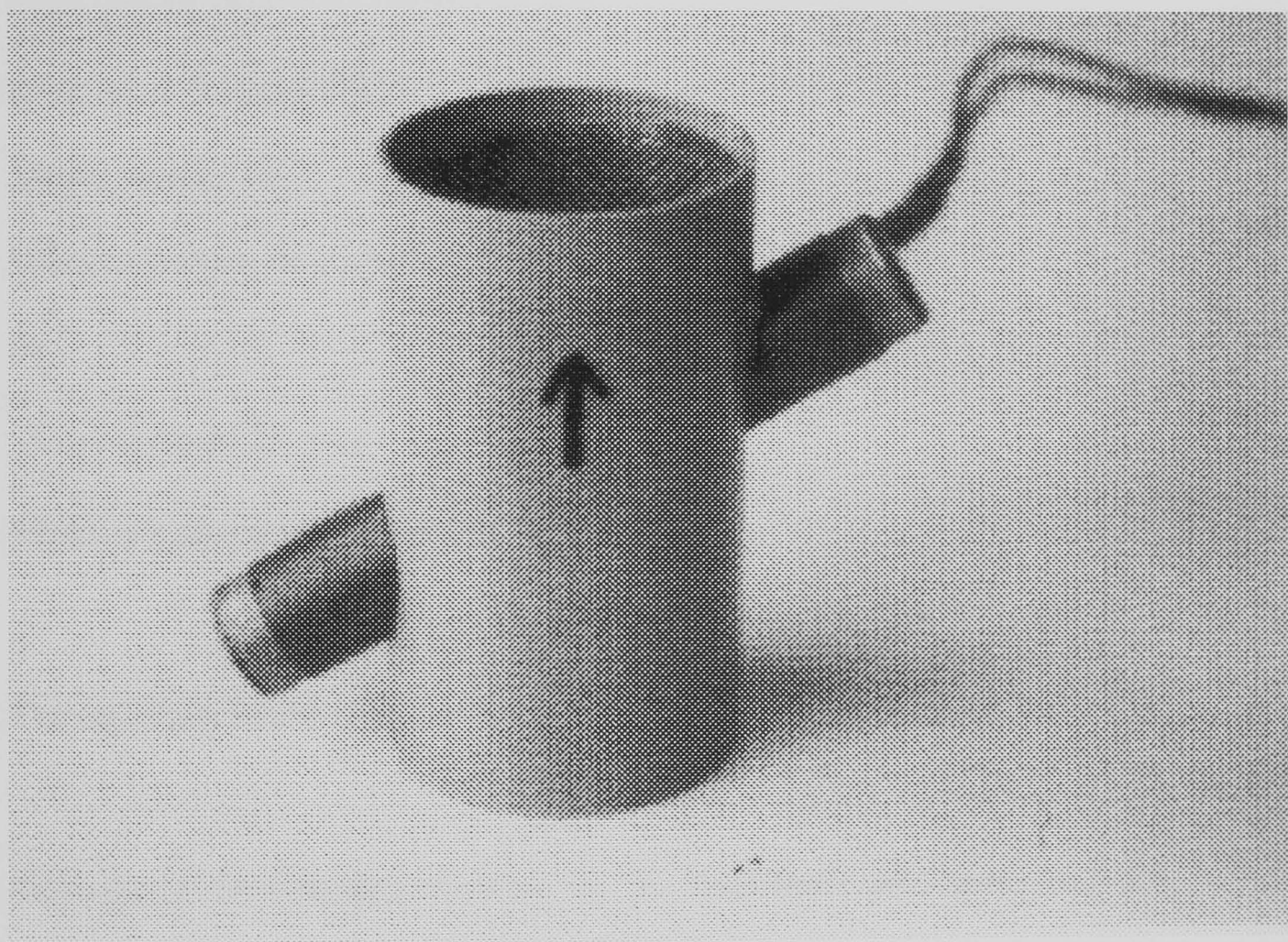
masses can be selected and the current produced by each ion beam sampled individually. An analogue signal is produced which is proportional to the dry gas concentration normalised to 100% ( $O_2 + CO_2 + N_2 + Ar = 100\%$ ).

Outputs from the flow tubes and mass spectrometer were fed into a custom built attenuator/amplifier for scaling. The output from this was split and fed simultaneously to a chart recorder and a DT2801, sixteen channel, twelve bit analogue to digital converter (Data Translation, Marlborough, USA) housed in a microcomputer (Compaq DeskPro 386<sup>TM</sup>, Compaq Computer Corporation, Houston, USA). The chart recorder provided a continuous hard-copy of data whilst allowing visual confirmation that signals were being recorded during the studies. Data acquisition and analysis were performed with the microcomputer using a programme written in Asyst (Asyst version 3.0, Macmillan Software Company, New York, USA) by Dr Lloyd Anderson and refined and modified by Dr David Marlin.

Data were acquired on four channels (Channel 1 : gas flow - right nostril; Channel 2: gas flow - left nostril; Channel 3:  $CO_2$  concentration; Channel 4:  $O_2$  concentration) at a frequency of 100Hz into ten second subfiles. Collection of data was under user control and sets of six subfiles (comprising one minute of data collection) were collected during exercise tests as detailed in Section (v) of this chapter.

Following collection, the programme was used to analyse five breaths from each selected subfile. The programme determined peak inspiratory and expiratory flow rates (PIF and

**Figure 3.2: Individual flow tube with ultrasound flow transducers fitted (arrow indicates direction of inspiratory flow).**



PEF), end expired  $\text{CO}_2$  and  $\text{O}_2$  concentrations ( $P_{\text{ETCO}_2}$  and  $P_{\text{ETO}_2}$ ) and inspiratory and expiratory times ( $t_i$  and  $t_e$ ). By integration of flow rates with time, inspired and expired volumes ( $V_i$  and  $V_e$ ) were obtained, whilst integration of the product of flow and gas concentrations allowed calculation of oxygen consumption and carbon dioxide production ( $\dot{V}_{\text{O}_2}$  and  $\dot{V}_{\text{CO}_2}$ ).

The following parameters were then calculated:

Tidal volume ( $V_T$ )

Respiratory frequency ( $f_b$ )

Minute volume ( $\dot{V}_e$ )

Mean inspiratory flow rate (MIF)

$t_i/t_e$

Mixed expired  $\text{CO}_2$  concentration ( $P_{\text{E}\text{CO}_2}$ )

Mixed expired  $\text{O}_2$  concentration ( $P_{\text{E}\text{O}_2}$ )

The algorithm for the analysis of respiratory data is shown in Figure 3.3.

Throughout the following chapters,  $V_i$ ,  $\dot{V}_e$ , respiratory dead space ( $V_d$ ) and alveolar ventilation ( $\dot{V}_A$ ) are expressed at body temperature and pressure saturated (BTPS) whilst  $\dot{V}_{\text{O}_2}$  and  $\dot{V}_{\text{CO}_2}$  are expressed at standard temperature and pressure dry (STPD).

Although the speed of sound ( $c$ ) varies with changes in gas composition and temperature, Buess, Pietsch, Guggenbuhl and Koller (1986) reported maximum changes in  $c^2$  (of which difference in transit time between the opposing signals is a function) during air breathing

to be around 3% and when ignoring these changes they obtained acceptable readings using an ultrasound flowmeter similar to the type used in these studies. In addition, using the same type of transducers as used in this study, it has been found that, for air breathing, the effects of change in gas composition and temperature oppose and substantially balance out (Innes, A., - personal communication). When  $V_i$  and  $V_e$  (both expressed at STPD) during canter in the studies of normal horses (Chapter Seven) were compared they were found to be similar. It is likely, however, that the next generation of ultrasound flow transducers will incorporate correction for changes in gas density.

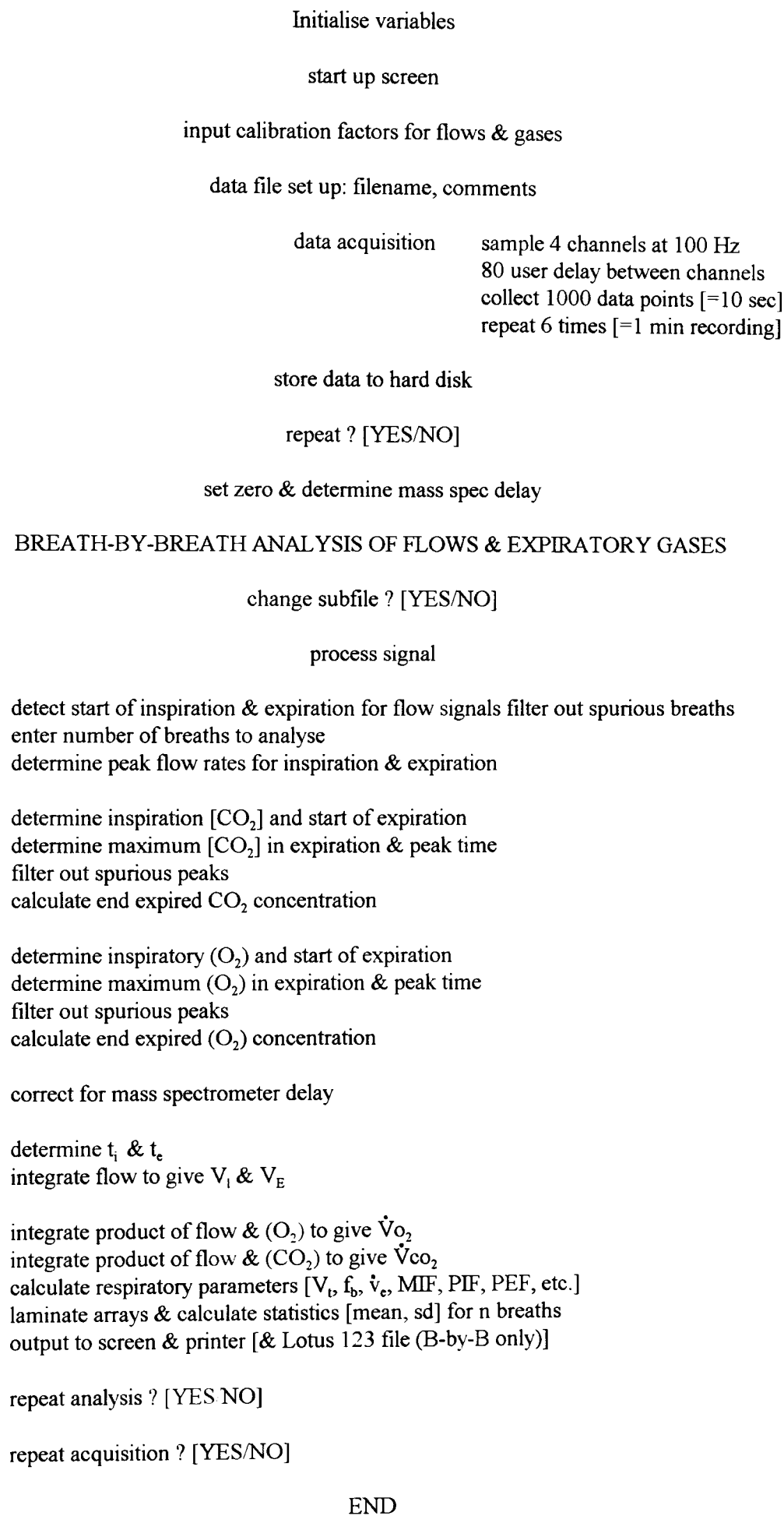
#### **(v) Sampling Protocol**

Respiratory flow rates, expired gas compositions and mixed venous blood temperature were recorded throughout the test on the six channel recorder, whilst data was acquired using the computer system at rest, during the last minute at each speed and throughout the canters.

At the beginning of each SET, whilst the horse was stationary on the treadmill, arterial and mixed venous blood samples were drawn simultaneously into heparinised syringes and a further mixed venous sample was taken and placed into fluoride/oxalate blood tubes whilst respiratory flow rates and gas compositions were recorded for one minute.

Heparinised blood samples (for blood gas, pH, oxygen contents and haemoglobin determination) were drawn carefully to avoid the introduction of air into the sample. After the samples were taken any gas bubbles were quickly expelled and the syringe was capped and placed on crushed ice and water until analysis could be carried out. Fluoride/oxalate

**Figure 3.3: Algorithm for acquisition and analysis of data from ultrasound flow transducers and mass spectrometer**



samples (for blood lactate measurement) were stored at -20°C until assayed.

The dead spaces of the sampling lines were drained immediately before samples were drawn. After sampling, the lines were flushed and filled with heparinised saline (10 iu heparin/ml).

Arterial and mixed venous blood samples were drawn simultaneously into heparinised syringes at the end of each period of exercise and after each minute of the canter. Additionally, mixed venous blood samples were drawn immediately before and two minutes after each canter and at the end of the test. Samples were handled similarly to those drawn at rest. The blood sampling protocol and parameters measured at each sampling point are shown in Figure 3.4.

#### **(vi) Analysis of blood samples**

##### *Blood gas and pH analysis*

Determination of arterial and mixed venous blood  $P_{O_2}$ ,  $P_{CO_2}$  and pH was performed within forty five minutes of sample collection using a micro blood-gas and acid-base balance analyser (ABL330; Radiometer). Immediately prior to analysis, samples were mixed thoroughly and the blood in the syringe tip expelled.



**Figure 3.4: Blood sampling protocol for Standardised Exercise Tests**

Sample	Blood Gas Analysis	O <sub>2</sub> Contents	[Hb]	Venous Blood Lactate
1. Rest	+	+	+	+
2. Walk + 10'	+		+	
3. Trot + 3'	+		+	
4. Walk + 5'	+	+	+	+
5. Canter + 1'	+		+	
6. Canter + 2'	+	+	+	
7. Walk + 2'				+
8. Walk + 10'	+	+	+	+
9. Canter + 1'	+		+	
10. Canter + 2'	+	+	+	
11. Walk + 2'				+
12. Walk + 10'	+	+	+	+
13. Canter + 1'	+		+	
14. Canter + 2'	+	+	+	
15. Walk + 2'				+
16. Walk + 10'	+	+	+	+
17. Canter + 1'	+		+	
18. Canter + 2'	+	+	+	
19. Walk + 2'				+
20. Walk + 10'	+	+	+	+

[Hb] = Haemoglobin concentration (determined on arterial blood)

Samples 17-20 apply only to training study and V3 challenge study

As these analyses were carried out at 37°C, it was necessary to correct results to blood temperature. The following equations were used for these corrections :

$$P_{CO_2}(t_v) = P_{CO_2} \times 10^{(0.021 \times (t_v-37))}$$

(Siggaard-Andersen, 1974)

$$P_{O_2}(t_v) = P_{O_2} \times 10^{(0.0252 \times \frac{1}{Po_2 \times 3.88} + 0.00564 \times (t_v-37))}$$

$$0.243 \times \frac{Po_2 \times 3.88}{100} + 1$$

(Severinghaus, 1966; Severinghaus, Stafford and Thunstrom, 1978;  
National Committee for Clinical Laboratory Standards, 1979)

$$pH(t_v) = pH + (0.0065 \times (7.4-pH)-0.0146) \times (t_v-37)$$

(Severinghaus, 1966)

Although these correction factors are based on human blood, it has been reported that their use in the horse provides results which are within ± 0.3 kPa and ± 0.01 pH unit of values obtained from tonometered horse blood determined at the same temperature (Butler *et al.*, 1991).

### Blood Oxygen Content

Blood oxygen content was measured by the method described by Tucker (1967) using a Clarke-type oxygen electrode in a water-jacketed glass chamber maintained at 37°C. The chamber contained a freshly-prepared solution of 0.69% potassium ferricyanide with 0.3% saponin and a small amount of octan-1-ol,n-octanol. The solution (which was completely

deoxygenated before use) released the oxygen from the blood allowing measurement with the oxygen electrode. The apparatus was calibrated before each SET with distilled water at 0°C which had been equilibrated with pure oxygen.

### *Haemoglobin*

Blood haemoglobin concentrations were determined on arterial blood samples, following blood gas determination, by a colorimetric method using a Coulter JT Automated Haematology Analyzer (Coulter Electronics Ltd., Luton). In this method, the absorbance of light at a wavelength of 525nm is measured for a sample of haemolysed blood. The calibration is checked using standards of known haemoglobin concentration. The absorbance is proportional to the haemoglobin concentration of the sample.

### *Blood Lactate*

Mixed venous samples drawn for blood lactate analysis were taken into fluoride/oxalate tubes and stored at -20°C. Blood lactate was determined by enzymic assay of perchloric acid extracts.

## **(vii) Validation of methods**

### *Linearity of response and repeatability of ultrasound flow transducers*

The linearity of response of the ultrasound flow transducers to a range of gas flow rates comparable to those encountered in exercising horses was investigated by measuring a series of known flows generated using variable flow vacuum cleaners. The test flow rates were set using rotameters (KDG Flowmeters, Burgess Hill). Rotameter measurements were made to the nearest 10 l.min<sup>-1</sup> (0.167 l.s<sup>-1</sup>).

The reproducibility of the flow transducers was investigated by passing a series of different flow rates ( $8.33\text{--}50.0\text{ l.s}^{-1}$  in  $8.33\text{ l.s}^{-1}$  steps) through each transducer and recording ten sets of one second's recording of flow rate for each level of flow.

#### *Determination of pressure-flow relationships of the flow tubes*

The pressure-flow relationships of the flow transducers were determined over the full range of flows encountered during inspiration and expiration using a pitot tube apparatus. Three determinations were made at each flow rate and the results meaned.

#### *Validation of measurements of flow and volume*

An anaesthetic ventilator (Bowring Medical Engineering Ltd., Oxford) directly coupled to a home-made wedge spirometer was used to pump known volumes of air ( $3\text{--}15\text{ L}$  in  $3\text{ L}$  steps) through a to-and-fro circuit into which the ultrasound flow transducers and a screen pneumotachograph were placed. The wedge spirometer was calibrated using  $3\text{ L}$  aliquots of air delivered from a calibrated gas syringe (Mercury Electronics [Scotland] Ltd). Ultrasound flow transducer and screen pneumotachograph signals were fed via an amplifier and analogue to digital (A/D) converter to the computer and recorded for off-line analysis. Flow signals from each measuring device were integrated using the Asyst programme to obtain values for gas volumes which were then compared.

#### *Determination of mass spectrometer transit time and response time*

The transit time (time for gas to pass along the sampling capillary to the mass spectrometer) and 90% response time for the mass spectrometer were determined using a microswitch and spring arrangement (Figure 3.5) to enable virtually instant switching of the mass

spectrometer sampling capillary from a stream of calibration gas (12% O<sub>2</sub> and 5% CO<sub>2</sub>, balance N<sub>2</sub>) to air whilst the mass spectrometer output was recorded on a chart recorder. The transit time and 90% response time could be determined from the chart recorder trace.

#### *Validation of method for measurement of $\dot{V}O_2$ during exercise*

To validate the method of determination of  $\dot{V}O_2$  against another, established method, the system was tested against an open flow system using human subjects. Although the exercising human does not show such high respiratory frequencies as the exercising horse, this was the only comparison of the two systems possible since a large enough open flow system for horses was not available. This study was carried out in collaboration with Professor P.J. Butler and Dr A.J. Woakes of the University of Birmingham.

Six people exercised on a bicycle ergometer whilst breathing through a rubber mouthpiece connected to a single flow tube with the mass spectrometer capillary in place. The flow tube was connected to a T-piece through which air was drawn at a known flow rate (measured by a rotameter - KDG Flowmeters, Burgess Hill), which exceeded the subjects' maximum inspiratory and expiratory flow rates. The oxygen content of the air passing through the system (comprising the subject's expirate plus the air drawn through from the atmosphere) was determined using a paramagnetic oxygen analyser (Servomex Ltd.).

Each subject exercised for two minutes at 25 Watts (W), followed by two minutes at 50W after which the work rate was increased by 50W every two minutes until the subject was unable to maintain the required work rate. Oxygen consumption was measured using both

systems during the last thirty seconds of each exercise step. A total of twenty nine simultaneous determinations of  $\dot{V}O_2$  were made.

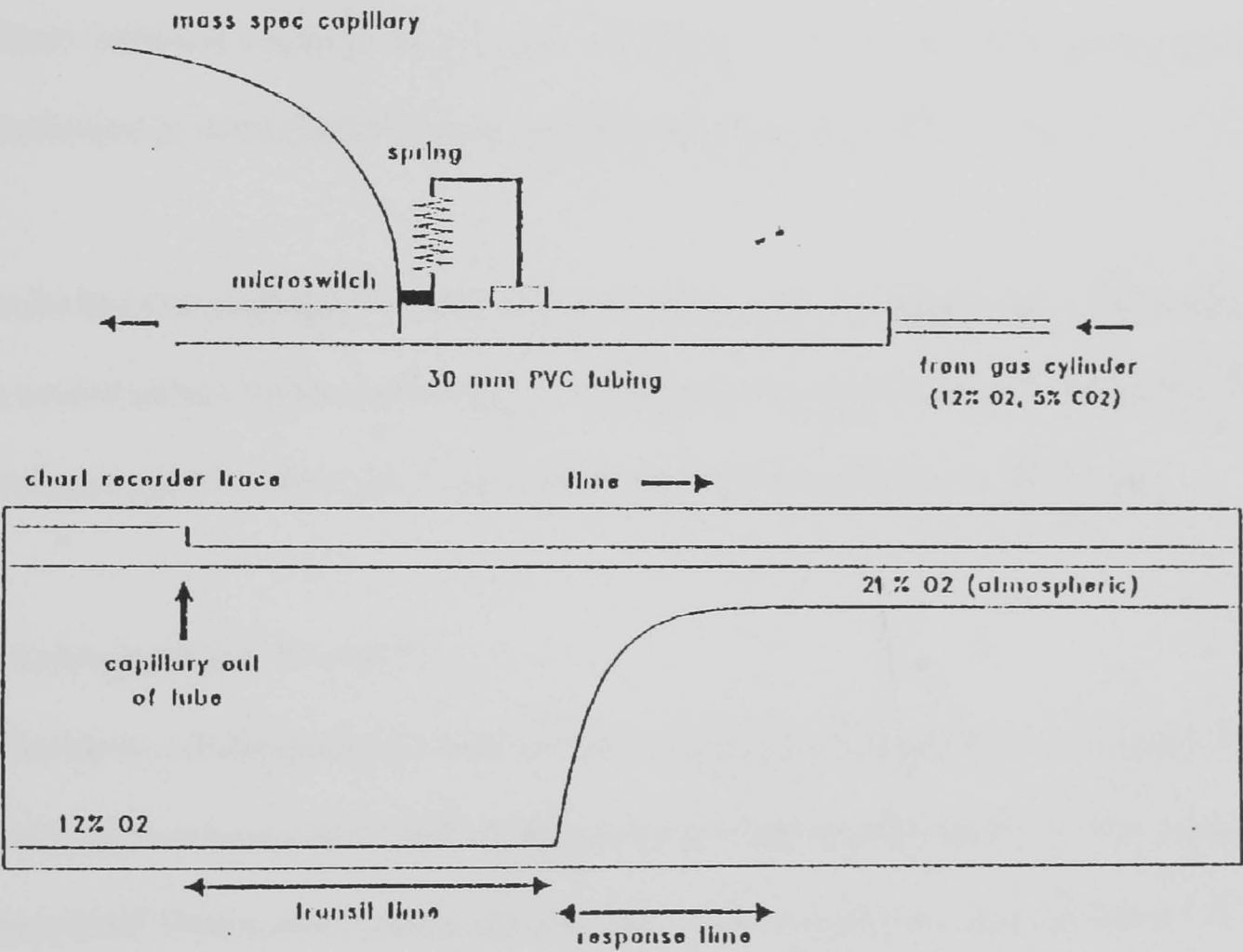
#### **(viii) Validation of site of sampling mixed venous blood**

As throughout these studies 'mixed' venous blood samples were drawn from the right atrium to avoid exercising referred patients with a pulmonary arterial catheter passing through the right atrioventricular and pulmonary valves, a comparison was made of  $PO_2$ ,  $P_{CO_2}$  and pH of blood drawn from the right atrium and the pulmonary artery (the traditional site of mixed venous sampling) simultaneously.

Four Thoroughbred horses, performing an exercise test as part of another project, were used in this study. The exercise protocol consisted of a ten minute walk ( $1.6m.s^{-1}$ ) followed by a continuous, incremental exercise test in which the horses cantered at speeds of six, eight, nine, ten, eleven and twelve  $m.s^{-1}$ , on a five degree incline for one minute at each speed, or until they were unable to continue further, followed by a fifteen minute walk.

Prior to exercise, an 8F diameter, percutaneous catheter introducer was located in the left jugular vein following aseptic preparation and local anaesthesia as described above in Section (iii). A 7F thermodilution catheter (Viggo, Swindon) was inserted via the introducer, passed along the jugular vein and positioned so that the proximal sampling port lay in the right atrium and the distal sampling port was situated in the pulmonary artery. Accurate location of the catheter in this position was achieved by observing the blood pressure trace at each port using a Gould pressure transducer attached to each port during placement.

**Figure 3.5: Determination of mass spectrometer transit time and 90% response time.** The sampling capillary is attached to the spring and microswitch, activation of the microswitch releases the tensed spring which pulls the capillary out of the calibration gas. A stylised version of the chart recorder trace is shown.



Paired blood samples were drawn from the two sites at rest, immediately prior to canter, at each canter speed, immediately following deceleration to walk, and at five, ten and fifteen minutes into the recovery walk. The sampling technique and method of determination of  $PO_2$ ,  $P_{CO_2}$  and pH were as described in Sections (v) and (vi) above.

### **(ix) Influenza Challenge Studies**

#### *General Considerations*

Three influenza challenge studies were carried out in which Thoroughbred horses were challenged by aerosol administration of influenza A/equi-2 (H3N8) virus.

In the first two challenges (V1 and V2), two horses were challenged with a third acting as a control animal. In the third study (V3), three horses were challenged with three others acting as controls, after which the control animals were themselves challenged.

#### *Challenge Studies V1 and V2*

The first two challenge studies were carried out using a similar protocol as follows. Three Thoroughbred horses which had not been vaccinated against influenza for at least two years were used. Data concerning the sex and ages of these horses are given in Table 3.1. The horses had not been in training for at least a year before entering the study. Serological examinations to determine the animals' serum antibody titres against equine influenza were performed using the single radial haemolysis (SRH) technique (Schild *et al.*, 1975) prior to the study and the horse in each trio with the highest titre was selected as the control animal.



The horses were housed for two weeks prior to pre-challenge testing. In addition to the SET, pre-challenge testing consisted of tracheal wash (TW) sampling and routine haematological and clinical chemistry screening to ensure that no signs of disease were present at the beginning of the study. In the first study (V1), the canter speeds used in the SETs were six, eight and ten m.s<sup>-1</sup>. As a result of this study it became apparent that unfit horses could exercise easily at these speeds, so in the second study (V2) speeds of eight, ten and twelve m.s<sup>-1</sup> were used.

Two weeks before challenge, the horses were moved to an isolated holding station approximately twenty five miles from Newmarket for the influenza challenge (this was necessary to avoid the possibility of infection spreading into the general horse population from the experimental animals). At the field station the horses were housed in loose boxes, bedded on paper and fed as at Newmarket, with feed levels being adjusted slightly as necessary to maintain body weight as constant as possible. After a two week period of acclimatisation, TW samples were taken the day before challenge.

Viral challenge was performed on day 0 using the method of Mumford, Hannant and Jessett (1990). The challenge virus was administered by aerosol using a Devilbiss nebuliser Model 65 (DeVilbiss, Pennsylvania, USA), 20ml of egg allantoic fluid containing 10<sup>7.3</sup> EID<sub>50</sub> of virus being nebulised over a thirty minute period in a closed stable (volume 56m<sup>3</sup>) containing both subject horses<sup>2</sup>. The stable was kept closed for an hour after nebulisation.

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<sup>2</sup>Using this method, 90% of the nebulised particles are in the respirable range (<5µm diameter). The antigen mass is distributed so that 80% of it is within particles within the respirable range (Hannant, D., personal communication).

A/equine-2 (H3N8) viral sub-types were used in both studies, the Newmarket/79 strain in the first and Suffolk/89 in the second. The change of strain between each study (as well as the one between these studies and the third challenge described below) was made to select strains, as they became available, to which the horses' previous vaccinations were likely to confer less immunity.

Following challenge the horses were returned to their loose boxes and remained there until returning to Newmarket on day 20 (the earliest time at which the horses could be safely brought back to Newmarket without risk of spreading influenza to the local horse population; Hannant, D., personal communication). The control animal was attended to (for feeding, watering, bedding and sampling) each day before the subject horses and after contacting the subject animals personnel did not return to the control horse until the following day.

A routine clinical examination was carried out and jugular venous blood samples drawn daily for the first 14 days after challenge for routine haematological examination. Nasopharyngeal swabs were taken daily for the first seven days post-challenge, placed in virus transport medium and stored at -70°C prior to submission for virus isolation. The effectiveness of challenge was further determined by serological examination of blood samples taken before and fourteen days after challenge. TWs were performed on days three, seven and fourteen after challenge.

Following the horses' return to Newmarket, SETs were carried out on day 21 post-challenge and TW samples were obtained at this time.

### *Challenge Study V3*

The third challenge study was carried out after the horses involved had undergone a period of training during which SETs were carried out at intervals to assess the effects of training on respiratory function during exercise. The training part of this study is described in Section (x).

The six horses, in full training at the conclusion of the training programme were divided into two groups on the basis of their serum SRH antibody titres to influenza. Following the final SETs carried out in the training study, the three animals with the highest SRH titres were taken to Lanwades Park, the Animal Health Trust's site outside Newmarket to act as control animals, whilst the three with the lowest titres were taken to the field station used in studies V1 and V2 for influenza challenge. The two groups were kept at different sites due to concerns as to the feasibility of keeping the control horses free of infection.

Prior to challenge, TW samples were obtained from both groups. Viral challenge was carried out as described for studies V1 and V2 (using the Suffolk/89 strain of influenza H3N8), except that in this study the horses were kept in the closed challenge box for two hours after nebulisation to increase the time of exposure to the challenge virus.

Following infection, the two groups were cared for, examined and sampled as in studies V1 and V2. Routine care, nasopharyngeal swabbing and blood sampling of the control group were carried out by other personnel to reduce the risk of human transfer of infection from the infected animals to the controls. TW samples were obtained by the author, but were

staggered 24 hours behind the treatment group and performed prior to visiting the latter animals.

Following return of both groups to Newmarket, sampling was continued as in studies V1 and V2, the control animals again being tested one day behind the treatment group to maintain similar intervals between measurements for the two groups.

SETs were performed on the control group on day 35 post-challenge, this group was taken to the field station where they underwent a challenge under the same conditions and with the same sampling protocol as for the treatment group in the first part of the study.

The canter speeds used in the SETs in this study were eight, ten and twelve  $\text{m.s}^{-1}$  and twelve  $\text{m.s}^{-1}$  on a three degree incline.

### **(x) Training Study**

This study was carried out to determine the way in which the respiratory parameters measured in the SET protocol are altered by the horse's state of fitness. Whilst a useful exercise in itself, this study was also carried out so that the likely effect of three weeks inactivity in challenge study V3 could be assessed. The training regime used was a similar one to that routinely used in many racing stables in this country and consisted of daily ridden exercise, six times a week (with one rest day) of gradually increasing intensity over a period of fifteen weeks.

Since it was necessary to produce six fully fit horses for challenge study V3, and the

incidence of lameness in horses undergoing training for high speed exercise is high (Jeffcott *et al.*, 1982; Evans, 1988), eight horses were put into the study to allow for the possibility of up to two animals being forced to drop out through injury.

SETs were carried out on all eight horses at the beginning of the study. For the first week of training, the horses were ridden out at walk only, for an average of 45 minutes daily. From the second week onwards, trotting was introduced in increasing amounts until at the end of the sixth week exercise consisted of two hours walking and trotting. In weeks seven and eight, the horses were cantered three times per week and this was increased to six days per week in weeks nine to eleven. From week twelve onwards fast work at gallop was introduced to the horses' exercise regime twice weekly.

SETs were performed in weeks six, eleven and sixteen, i.e. at the end of the period of solely walking and trotting exercise, after the period of cantering and following completion of the training regime. Canter speeds of eight, ten, and twelve  $\text{m.s}^{-1}$  were used in all SETs whilst in the final test an extra step was added involving canter at twelve  $\text{m.s}^{-1}$  with the treadmill set at a three degree incline.

#### **(xi) Referred Cases**

Thirteen referred cases were accepted from veterinary surgeons in practice for exercise testing. Thoroughbred horses only were accepted in which the major presenting signs were loss of performance or poor exercise tolerance and the owner or trainer was willing to allow the horse to undergo exercise testing.

Upon admission, all horses underwent a normal clinical examination, including a gait evaluation, as well as routine analysis of blood samples taken at rest for haematology and clinical chemistry (serum creatine phosphokinase [CPK], serum aspartate aminotransferase [AST], serum gamma glutamyl transferase [GGT], total protein and albumin; serum protein electrophoresis). Endoscopic examinations were carried out before and after fast, ridden exercise to evaluate upper respiratory tract function. At this time it was also possible to listen to the horse during exercise to determine the presence of adventitious respiratory sounds. Resting electrocardiographs were recorded and, in some cases, exercising videoendoscopy was also carried out.

Familiarisation to treadmill exercise was carried out over a period of a few days, between four and six sessions being required. At the end of this period horses exercised in a balanced and relaxed fashion, their heart rates at rest, walk, trot and canter on the treadmill being similar to those of more experienced experimental animals at the same speeds.

SETs were carried out as described above, with canter speeds of eight, ten and twelve  $\text{m.s}^{-1}$ , with TW samples taken after the test.

## CHAPTER FOUR - SYSTEM VALIDATION

Although aspects of the system<sup>1</sup> used for measurement of respiratory function have been described previously (Woakes, Butler and Snow, 1987) and it has been used to measure responses of the Thoroughbred horse to exercise (Anderson *et al.*, 1989; Anderson *et al.*, 1990; Art *et al.*, 1990; Butler *et al.*, 1991; Young *et al.*, 1992; Butler, Woakes, Smale, Roberts, Hillidge, Snow and Marlin, 1993a; Butler, Woakes, Anderson, Roberts and Marlin, 1993b), certain facets have not previously been adequately investigated. This chapter describes various validation studies which have been performed to characterise further the system and its effect on the horse.

### Summary of methods

The linearity of response of the ultrasound flow transducers to a range of flows comparable to those encountered in exercising horses was investigated by measuring a series of known flows (set using rotameters), generated using variable flow vacuum cleaners. Measurements were made to the nearest 10 l.min<sup>-1</sup> (0.167 l.s<sup>-1</sup>). The repeatability of the A/D conversion for air flow rates was tested by passing flow rates of 8.33-50.0 l.s<sup>-1</sup> (in 8.33 l.s<sup>-1</sup> increments) through the flow transducers, coupled in series. Ten sets, of one second's recording each, were collected at each flow rate.

The accuracy of the flows was assessed by comparison with a screen pneumotachograph. A ventilator coupled to a wedge spirometer was used to pass air to-and-fro through the

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<sup>1</sup>i.e. lightweight facemask, ultrasound flow transducers, mass spectrometer and dedicated computer analysis programme.

flow tubes and pneumotachograph. Signals were integrated using the Asyst computer programme to give values for flow in each direction analogous to inspiratory and expiratory measurements.

The pressure-flow relationship of the flow tubes was determined for their full range using a pitot tube apparatus.

The delay time and 90% response time for the mass spectrometer were determined by using a microswitch and spring arrangement to enable virtually instant switching of the mass spectrometer sampling capillary from a stream of calibration gas (12% O<sub>2</sub>; 5% CO<sub>2</sub>) to air whilst recording the mass spectrometer output on a chart recorder. Data presented for the latter two validations (pressure-flow relationship of the flow tubes and mass spectrometer delay and response times) were collected by Dr David Marlin. The interpretation of these data presented below is, however, the author's.

The accuracy of the computer algorithm for calculation of  $\dot{V}O_2$  using signals from the ultrasound flow transducers and mass spectrometer was determined by comparison with a flow through system using six human subjects and a bicycle ergometer. This study was performed collaboratively with Professor P.J. Butler and Dr A.J. Woakes of the University of Birmingham.

The methods used are described in more detail in Chapter Three, Section (vii).



**Results**

*(i) Response of the flow transducers*

The response of each flow transducer over the range  $-60 \text{ l.s}^{-1}$  to  $+60 \text{ l.s}^{-1}$  was linear (Figure 4.1). The response did not differ between the two transducers.

The relationship between air flow rate and the digital value, calculated by the A/D converter for each flow transducer were as follows:

Right flow transducer:  $\text{A/D value} = 2042 + 9.04V$

Left flow transducer:  $\text{A/D value} = 2044 + 9.07V$

where  $V = \text{flow rate (l.s}^{-1}\text{)}$ . In both cases,  $r = 1.0$  ( $p < 0.001$ ).

Values for repeated measurements of air flow rates in the range  $8.3\text{-}50.0 \text{ l.s}^{-1}$  are shown in Table 4.1.

*(ii) Pressure-flow relationships of the flow tubes*

Figure 4.2 shows the pressure-flow relationship of the flow tubes for flows in both the 'inspiratory' and 'expiratory' directions. At a flow rate of  $50 \text{ l.s}^{-1}$ , mean pressure was  $2.6 \text{ cm H}_2\text{O}$  ( $\pm 0.2$ ).

The relationship of pressure to flow was described by the following equation:

$$\text{Pressure (cm.H}_2\text{O)} = -0.30457 + 0.17915V + 0.00054V^2 + 0.00015V^3$$

where  $V = \text{flow rate (l.s}^{-1}\text{)}$ .  $r = 0.997$  ( $p < 0.001$ ).

Figure 4.1: Relationship between airflow rate and A/D converter digital value for each flow transducer.

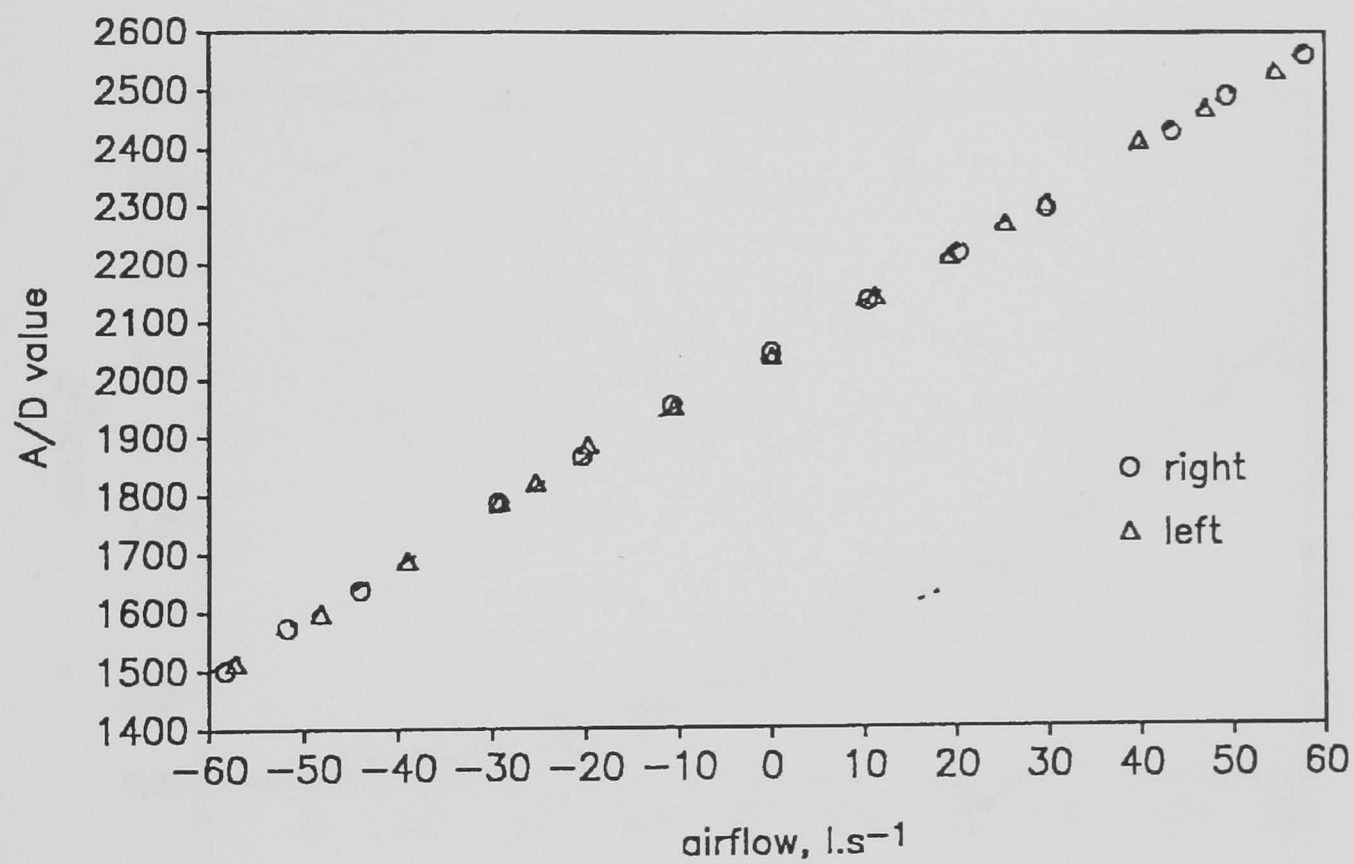
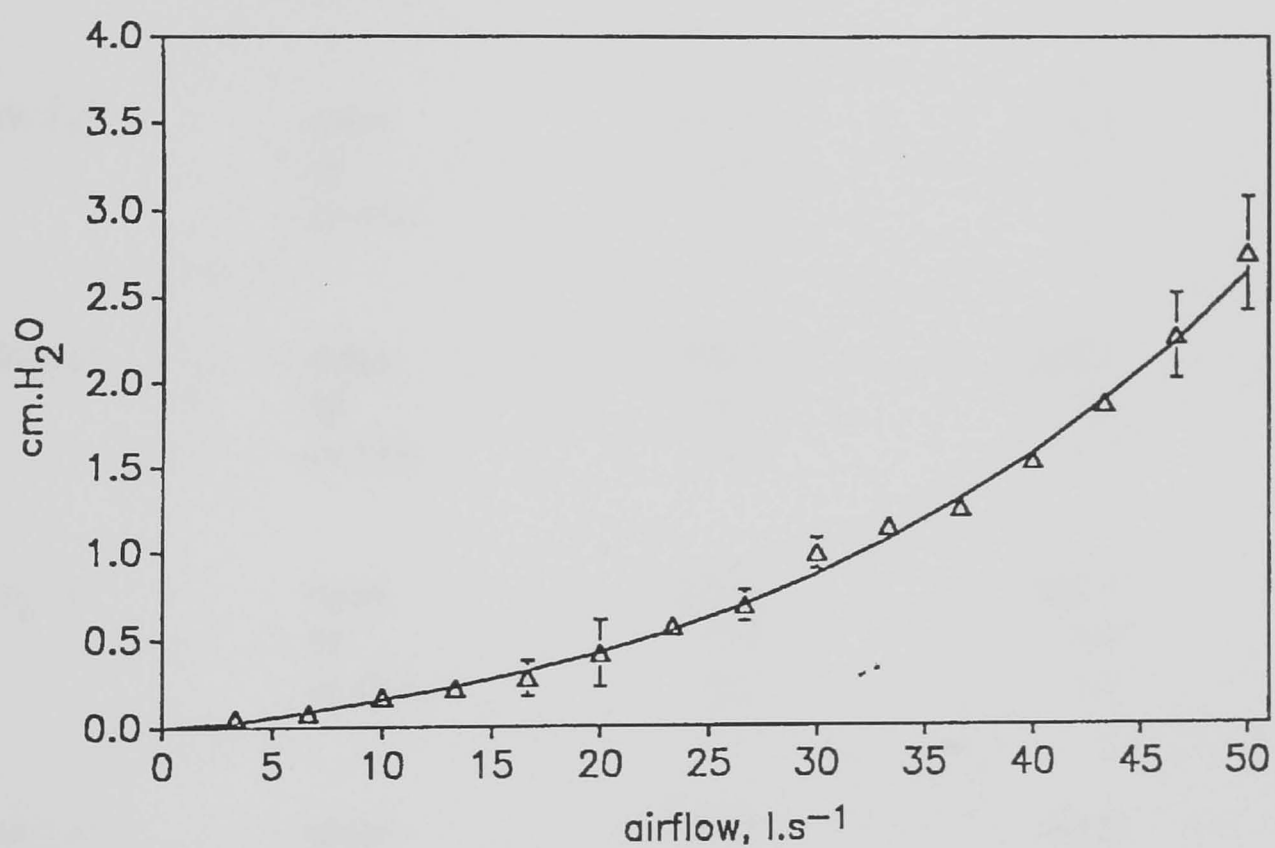


Figure 4.2: *In vitro* pressure-flow relationship of the flow tubes as measured using a pitot tube.



**Table 4.1: Reproducibility of A/D converted digital value for air flow rates obtained with ultrasound flow transducer analysed using the *Asyst* computer programme for each flow transducer. Ten determinations at each flow rate.**

Transducer:		Left side	Right side
Test flow rate (from rotameter)			
8.33 l.s <sup>-1</sup>	mean:	62.0	78.3
	sd:	4.4	6.5
	cv (%):	7.1	8.3
16.66 l.s <sup>-1</sup>	mean:	137.2	154.3
	sd:	6.8	5.7
	cv (%):	5.0	3.7
25.00 l.s <sup>-1</sup>	mean:	208.7	228.8
	sd:	6.1	3.5
	cv (%):	2.9	1.5
33.33 l.s <sup>-1</sup>	mean:	272.9	309.5
	sd:	5.9	4.4
	cv (%):	2.2	1.4
41.66 l.s <sup>-1</sup>	mean:	350.3	376.6
	sd:	5.5	8.3
	cv (%):	1.6	2.2
50.00 l.s <sup>-1</sup>	mean:	423.1	452.5
	sd:	5.7	5.3
	cv (%):	1.4	1.2

Values reported are the mean of ten determinations

SD=standard deviation

CV=coefficient of variation

### **(iii) Validation of measurements of flow and volume**

The values for volume obtained by integration of the flow signals for the ultrasound flow transducers and pneumotachograph are compared in Figure 4.3. The overall error for each of the ultrasound flow transducers was 3.6% ( $\pm 2.5$ ) and 2.8% ( $\pm 2.2$ ), compared with 3.0% ( $\pm 1.6$ ) for the pneumotachograph.

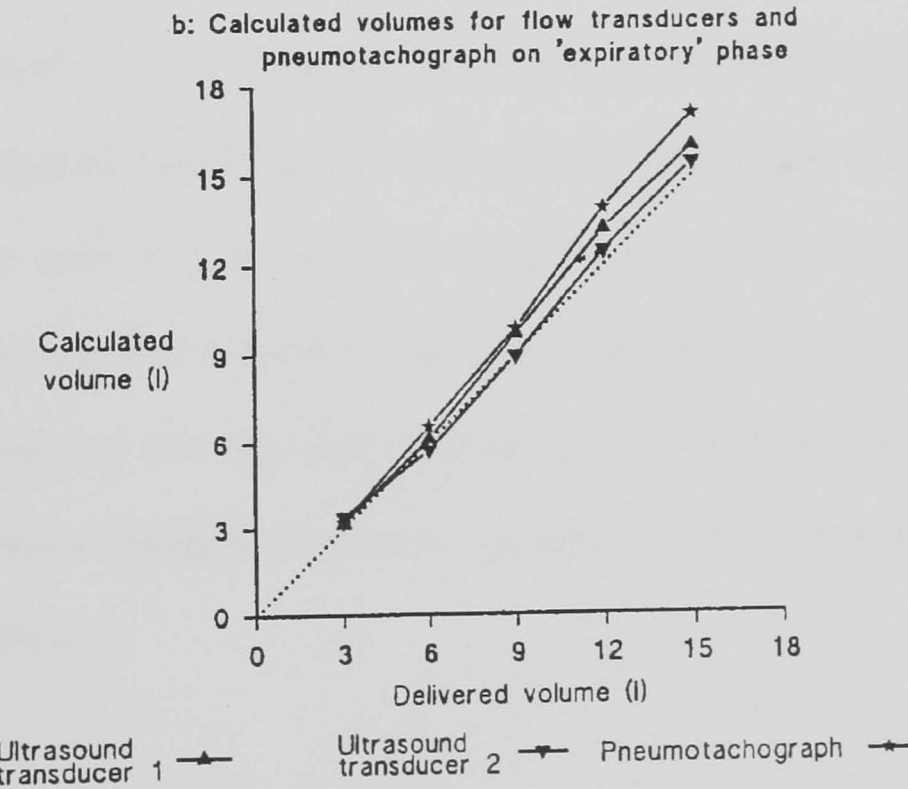
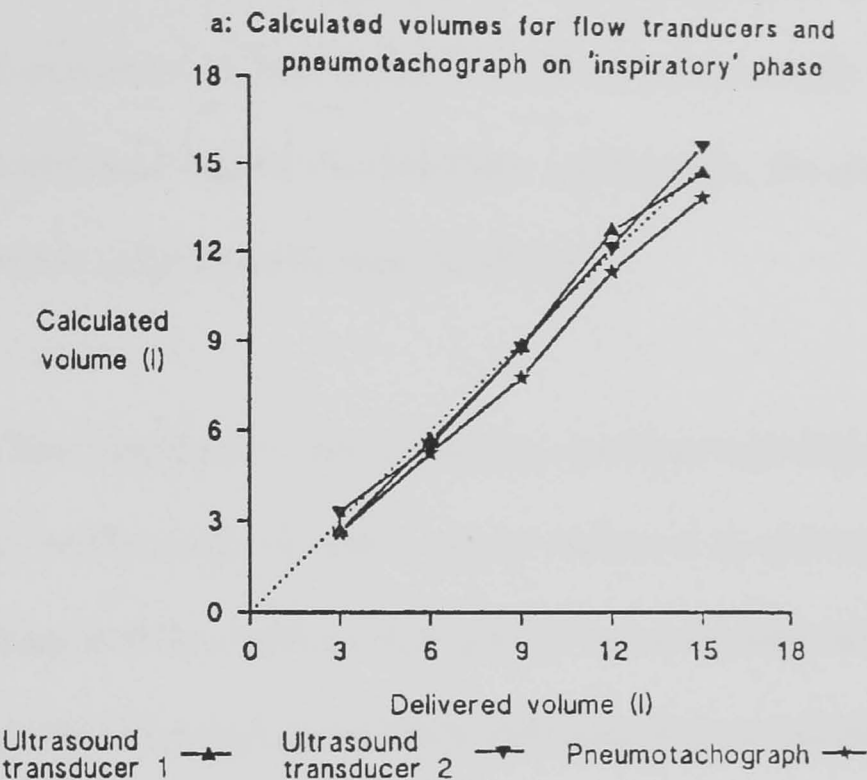
### **(iv) Determination of mass spectrometer transit time and 90% response time**

The mean transit times for oxygen and carbon dioxide were 247.8ms (SEM $\pm 2.4$ ) and 245.5ms ( $\pm 2.4$ ) respectively. The difference between transit times for the two gases was highly significantly different ( $p < 0.001$ ). The 90% response times for oxygen and carbon dioxide were 51.8ms ( $\pm 0.8$ ) and 32.8ms ( $\pm 0.9$ ) respectively, which were, again, highly significantly different ( $p < 0.001$ ).

### **(v) Validation of measurements of $\dot{V}O_2$**

As reported separately in Butler *et al.* (1993a), when the values obtained using the ultrasound flow transducers and computer programme were plotted against those obtained using the flow through method, the intercept was not significantly different from zero ( $-0.046 \pm 0.078$ ), nor was the slope ( $0.954 \pm 0.047$ ) significantly different from unity ( $p < 0.05$ ).

Figure 4.3



## Discussion

### *System validation*

Although Woakes, Butler and Snow (1987) described the system used in these studies for measurement of respiratory variables during exercise, they did not fully validate the system against other, established methods of measurement. Additionally, the computer programme for data analysis has not previously been validated.

The ultrasound flow transducers were found to give a linear response over a range of  $-60 \text{ l.s}^{-1}$  to  $+60 \text{ l.s}^{-1}$ , as this range of airflow can be measured at each nostril, this gives the capacity to measure total flow rates in excess of  $100 \text{ l.s}^{-1}$  (assuming fairly even distribution of flow between the two nostrils) and hence sufficient range to encompass the peak flow rates associated with strenuous exercise<sup>2</sup>.

The coefficient of variation of the A/D conversion of flow transducer signal voltage to digital value ranged between 1.2 and 8.3% (Table 4.1). The greatest variation occurred at the lower flow rates ( $8.33$  and  $16.66 \text{ m.s}^{-1}$ ) and is very likely to have been due to a genuinely greater variation in the flow since it was considerably more difficult to stabilise air flow at these rates (the rotameter reading was noticeably more varied during these measurements than at the higher flow rates). The response of the two transducers was not significantly different.

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<sup>2</sup>Peak expiratory flow rates slightly greater than  $100 \text{ l.s}^{-1}$  were reported by Attenburrow, Flack and Portergill (1990), deducing flow rate from respiratory sound intensity. Gillespie (1974) reported maximal forced expiratory flow rates of  $80\text{-}100 \text{ l.s}^{-1}$  in anaesthetised horses and similar rates in exercising horses (Gillespie, 1975).

A comparison of calculated volumes of air flow obtained by integration of signals from the ultrasound flow transducers and pneumotachograph showed no significant difference between the two instruments, the overall errors for the ultrasound flow transducers being 3.6% ( $\pm 2.5$ ) and 2.8% ( $\pm 2.2$ ).

Values for  $\dot{V}O_2$  obtained using the present system were in good agreement with those using the flow through system. Although it was necessary to use human subjects for this comparison (due to the unavailability of a flow through system large enough for equine use), the gain of the A/D converter was adjusted so that the digital values produced were similar to those obtained from measurements of exercising horses. Since  $\dot{V}O_2$  is calculated by the computer programme using values of gas flow rates (obtained using the ultrasound flow transducers) and fractional expired oxygen concentrations (determined by mass spectrometry), and since it is essential to match the flow transducer and mass spectrometer signals accurately in time (see below), the close agreement of the values for  $\dot{V}O_2$  using the two systems was reassuring.

### *Effects of respiratory measurements on respiration*

Measurements of respiratory function may be affected by the equipment used to obtain them. In man, different types of masks and mouthpieces have been found to alter respiration at rest (Gilbert, Auchincloss, Brodsky and Boden, 1972; Askanazi, Silverberg, Foster, Hyman, Milic-Emili and Kinney, 1980; Newton, Hamilton and Forster, 1983). In the horse various respiratory masks have been evaluated for their effect on ventilation and gas exchange.



Systems incorporating one way valves to allow measurement of ventilation volumes have been shown to affect the pattern of breathing, for example, Bisgard *et al.* (1978) found a significant reduction in breathing frequency ( $f_b$ ) and increase in tracheal pressure swings in ponies during exercise at  $2.22 \text{ m.s}^{-1}$  whilst wearing such a system. Further, Bayly *et al.* (1987b) and Gauvreau, Young, Wilson, Staempfli and McDonell (1993) found an exacerbation of exercise-induced hypoxaemia and hypercapnia associated with the use of valve systems. Evans (1987) reported effects on both  $f_b$  and blood gases and Art and Lekeux (1988) alterations in  $f_b$  and oesophageal pressure.

Although flow through systems were found to interfere less with respiration, even they affected  $P_a\text{CO}_2$  unless flow rates in excess of  $6300 \text{ l.min}^{-1}$  were used (Bayly *et al.*, 1987b). Landgren, Gillespie and Leith (1991) reported substantial breathing loads associated with the use of a flow through system and pneumotachographs.

The presence of a mask may alter respiration due to mask resistance increasing the work of breathing, the psychological effect of the mask over the face or rebreathing of expired gas due to an increased dead space ( $V_d$ ) (Harkins, 1992). Of these possible mechanisms, it appears unlikely that psychological factors are of importance for most systems in the horse as Harkins (1992), using a face mask with ultrasound flow transducers reported blood gas values similar to control data and normal 1:1 respiratory:locomotory coupling in horses wearing an open mask<sup>3</sup>.

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<sup>3</sup>This mask was a 'cut away' version of one which, with 5.2 cm flow tubes housing ultrasound flow transducers, was found to affect these parameters of respiration and to cause increased upper airway pressures.

In this laboratory it has been found that horses adapt well to wearing the facemask and after one or two runs wearing it have heart rates comparable to exercise with no mask. It has also been noted that stride rate at a given speed is unaffected by the presence of the mask, even in horses only recently accustomed to treadmill exercise. Whether systems involving heavy equipment or long lengths of tubing may have a psychological effect on the horse's pattern of breathing is as yet uncertain.

The additional dead space inherent in any mask system may be of great importance in the effect of the mask on respiration as Harkins (1992) found that the use of a bias flow of 50 l.s<sup>-1</sup> to prevent rebreathing of expired mask dead space air resulted in blood gas values during exercise not significantly different to those with no mask. In addition, when the bias flow was used, 1:1 respiratory:locomotory coupling was restored.

It has been shown in an earlier study (Butler *et al.*, 1993a) that the face mask fits the contours of horses' heads well with the result that leakage of air around the seal does not represent an important source of error (see Chapter Three, Section (iii)). Earlier work, using a cadaver head also showed that upper airway pressure and resistance was not changed by the mask and flow tubes when air flow rates up to 23.7 l.s<sup>-1</sup> were used (Young, S., personal communication).

In the present studies the pressure change across the flow tubes, 2.6 cm.H<sub>2</sub>O at air flow rates of 50 l.s<sup>-1</sup> was not large. Bayly *et al.* (1987b) reported similar pressures for flow through apparatus and greater ones for valve systems, whilst Gauvreau *et al.* (1992) found pressure drops of 2.2 and 1.0 cm H<sub>2</sub>O across ultrasound flow meters and valves

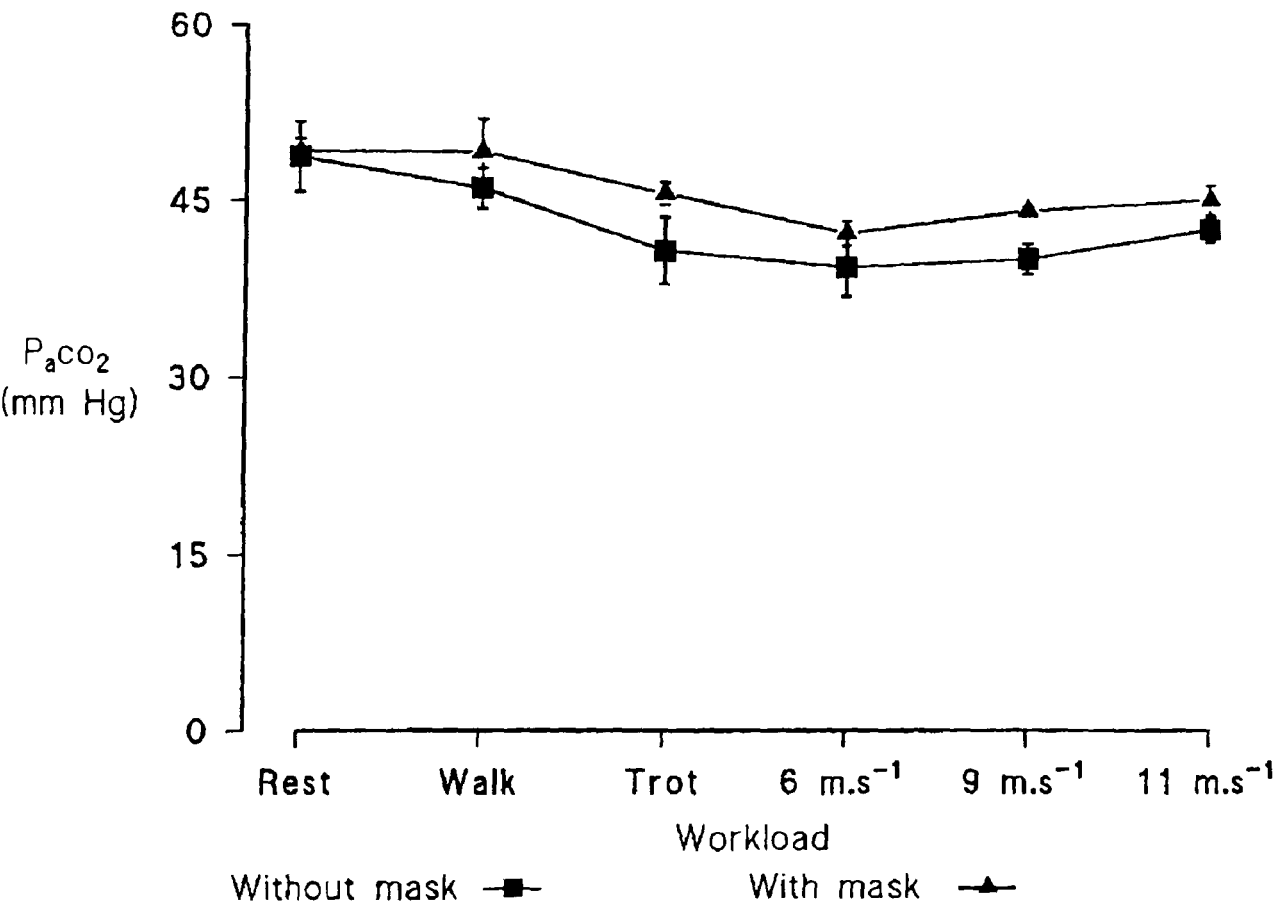
respectively at a flow rate of 70 l.s<sup>-1</sup>.

The effect of this mask on blood gases was earlier determined by exercising three horses with and without the mask and measuring arterial and mixed venous (right atrial) Po<sub>2</sub>, Pco<sub>2</sub> and pH (Smale, 1991). The only significant effect of the mask was a slight increase in P<sub>a</sub>co<sub>2</sub>, which was 3 mm Hg (±1.5) higher when the mask was worn during exercise (Figure 4.4).

Other workers have reported effects of respiratory masks on blood gases. Gauvreau *et al.* (1992) found significant carbon dioxide retention during exercise and Harkins (1992) alterations in blood gases when horses exercised wearing masks, in the former case using a valve system and in the latter without. Pan, Forster, Bisgard, Kaminski, Dorsey and Busch (1983) also reported carbon dioxide retention in ponies exercising whilst wearing a mask.

Evans (1987) reported considerably greater effects on blood gases using his valve system. During exercise on a 6° incline, P<sub>a</sub>co<sub>2</sub> was 5.4 mm Hg higher with the mask at 4.5 m.s<sup>-1</sup> rising to 10 mm Hg higher at 9 m.s<sup>-1</sup>. P<sub>a</sub>o<sub>2</sub> was lower with the mask, by as much as 10-14 mm Hg at 9 m.s<sup>-1</sup>. In comparison to these other findings, the effect on blood gases of the system used in the present studies is minor. Bayly *et al.* (1987b) found that valve systems caused an exacerbation of exercise-induced hypoxaemia and hypercapnia, whilst flow through systems increased exercising P<sub>a</sub>co<sub>2</sub> unless flow rates as high as 6300 l.min<sup>-1</sup> were used.

**Figure 4.4:** A comparison of  $P_{aCO_2}$  (mm Hg) in Thoroughbred horses exercising with and without the facemask (data from Smale, 1991).



The system under evaluation has been shown to be well-tolerated and practical for use in measurement of respiratory variables in the exercising horse with sufficient accuracy and comparatively little interference with respiration.

Compared to systems using valves and screen pneumotachographs, the resistance of the system is small, with an accordingly lesser effect on respiration. In addition, when a horse was exercised with an ultrasound flow transducer over one nostril and a pneumotachograph over the other, it was found that after 25 minutes exercise (including five minutes at trot or slow canter), the calibration of the ultrasound flow transducer was unchanged whereas the pneumotachograph calibration had changed considerably due to accumulation of water and respiratory secretions. Although flow through systems with high flow rates appear not to interfere significantly with breathing, they are limited in the range of variables which they can measure.

The ensuing chapters will describe studies of respiration in exercising horses using the system described.

#### *The computer programme for data analysis*

Before leaving this Chapter, some further consideration of the computer programme for analysis is appropriate. The programme was originally written, in this laboratory, specifically for use with the ultrasound flow transducers and mass spectrometer, by Dr Lloyd Anderson and was considerably refined and modified by Dr David Marlin. The algorithm for calculation of respiratory variables was shown in Chapter Three, Section (iv).

Similar programmes have been written for use in man, for example, Beaver, Wasserman and Whipp (1973), Riblett (1983) and Noyes (1985); the latter two authors using a system originally developed for breath-by-breath measurement of  $\dot{V}O_2$  and  $\dot{V}CO_2$  in exercising calves (Creel, 1980).

An important aspect of such programmes is the alignment of the flow transducer signals with those of gas concentration since the latter will lag the former due to the transit time of gas through the sampling capillary into the mass spectrometer. A further, smaller, error will occur if the response time of the mass spectrometer is not accommodated into calculations.

Various authors have addressed the problem of correcting calculations of gas exchange for mass spectrometer response time (Mitchell, 1979; Arieli and Van Liew, 1981; Bates, Prisk, Tanner and McKinnon, 1983). Mitchell (1979) reported a 20% underestimation of gas exchange rate if analyser response time is not taken into consideration. Bates *et al.* (1983) suggested that although more complex methods of correction, such as exponential model methods or Wiener filtering give the most accurate results, a simple time-shift correction removes the majority of the error<sup>4</sup> and is likely to be sufficient for most applications.

The mass spectrometer transit time quoted by the manufacturers of the *Airspec 2000* used in these studies is  $\approx 200$  ms. In most of the studies described in succeeding chapters, the total delay (and hence the correction factor) calculated by the computer programme using

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<sup>4</sup>producing a 10-fold reduction in error for these workers.

the abrupt decrease in carbon dioxide concentration at the commencement of inspiration, was fairly constant at 250-260 ms. The transit time is dependent on the length and diameter of the mass spectrometer capillary tube, but is also affected by respiratory secretions which may impede passage of gas along the capillary and increase transit time. Although it varied relatively little, it is important that the delay is calculated for each individual breath as using an averaged value may reduce the accuracy of calculated values for  $\dot{V}O_2$  and  $\dot{V}CO_2$ .

In breath-by-breath determination of  $\dot{V}O_2$  and  $\dot{V}CO_2$  during exercise, rapid changes in gas concentration must be followed since when  $f_b$  is of the order of  $120 \text{ min}^{-1}$ , expiratory time is only around 250 ms. The 90% response times for oxygen and carbon dioxide (51.8 and 32.6 ms respectively) would appear sufficiently fast to follow these changes quickly enough for estimates of  $\dot{V}O_2$  and  $\dot{V}CO_2$  to be reasonably accurate. Similarly, the ultrasound flow transducer frequency response is adequate to follow the changes in gas flow during respiration at high speeds. The frequency response quoted by the manufacturers is 80Hz and the response has been checked up to 100Hz using a signal generator (Marlin, D., personal communication). This assertion is supported by the close agreement between  $\dot{V}O_2$  measured using the mass spectrometer and using the flow through method, although admittedly  $f_b$  in the human subjects was not as high as in cantering horses. Nevertheless, there will be a slight underestimate due to the concentrations of oxygen and carbon dioxide in expired gas changing slightly faster than the mass spectrometer can follow and this is not accounted for by the simple transit time correction applied. Since the response for oxygen is slightly slower than for carbon dioxide,  $\dot{V}O_2$  will be underestimated slightly more than  $\dot{V}CO_2$  and hence respiratory exchange ratio will be slightly overestimated. This error is likely to be small and insignificant in comparative studies using the same system.

## CHAPTER FIVE - VALIDATION OF SITE OF SAMPLING OF MIXED VENOUS BLOOD

A further, short, validation study was necessary to confirm the suitability of the right atrial sampling site for mixed venous blood during exercise.

### Summary of Methods

As described in Chapter Three, Section (viii), blood samples were simultaneously drawn from the pulmonary artery and right atrium of four Thoroughbred horses undergoing an incremental exercise test (as part of another study).

### Results

A total of 42 pairs of samples were collected and analysed. Figures 5.1-5.3 show the relationship between values for  $P_{O_2}$ ,  $P_{CO_2}$  and pH obtained from each site.

For all variables, there was no significant difference between samples drawn from the two sites ( $p < 0.001$ ). Correlation coefficients for  $P_{O_2}$ ,  $P_{CO_2}$  and pH were 0.996, 0.998 and 0.997, respectively. The relationship between values from the two sites were described by the following equations:

$$P_{(pa)}O_2 = 0.983684(P_{(ra)}O_2) + 0.694088$$

$$P_{(pa)}CO_2 = 1.0265(P_{(ra)}CO_2) - 1.0462$$

$$pH_{(pa)} = 1.0093(pH_{(ra)}) - 0.070164$$

where the suffixes  $_{(pa)}$  and  $_{(ra)}$  denote pulmonary arterial and right atrial values respectively.

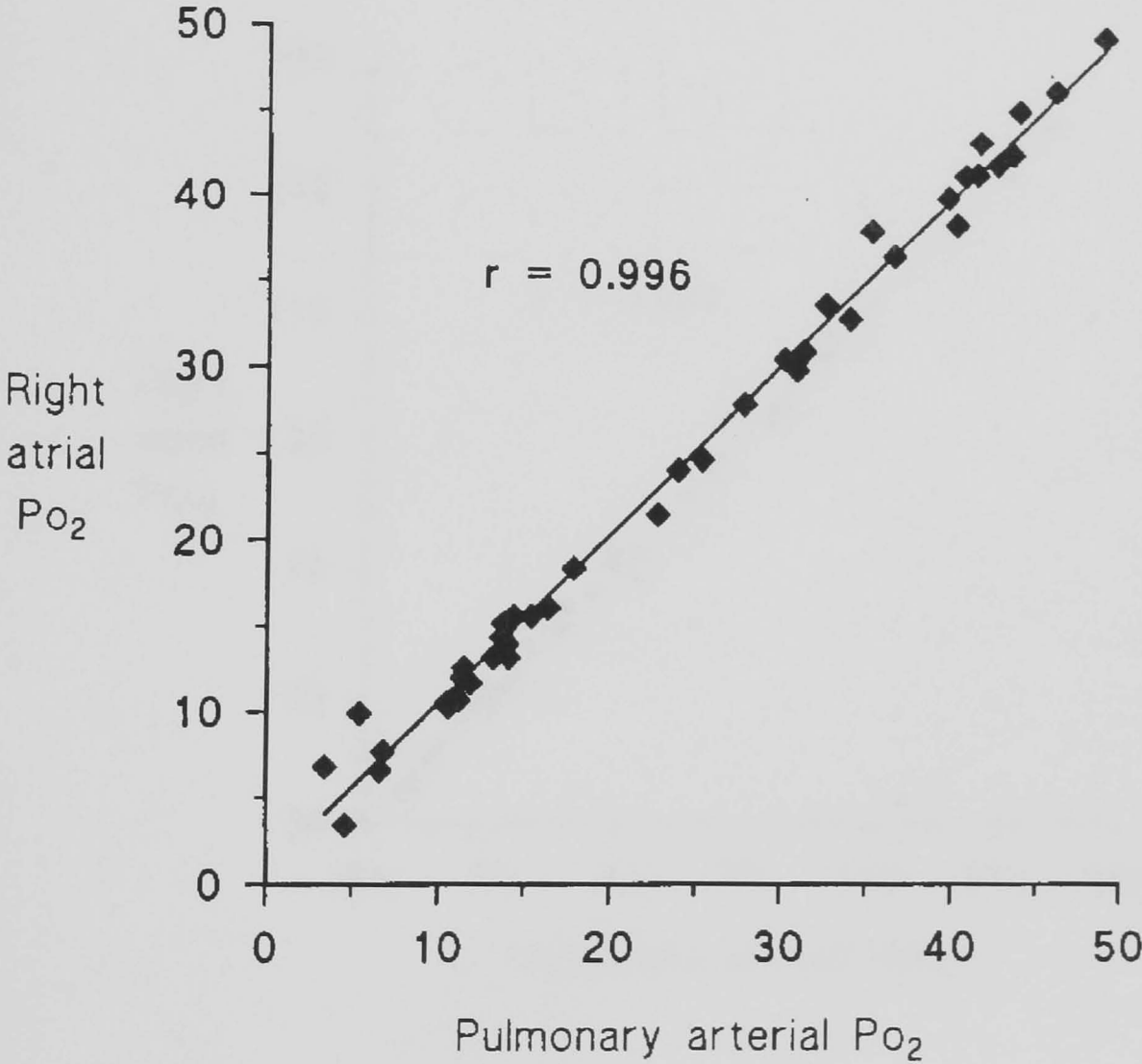


## Discussion

The usual site for mixed venous blood sampling is the pulmonary arterial trunk to ensure that samples are representative of blood draining all parts of the body, blood at this point having been thoroughly mixed in passage through the right heart. For sampling of referred clinical cases it was considered desirable to sample from the right atrium if possible, thus avoiding the need to exercise patients with a catheter passing through the right atrioventricular and pulmonic valves. Although Milne, Muir and Skarda (1975) found no difference between the two sites for  $P_{O_2}$ ,  $P_{CO_2}$  or pH in resting horses, it was necessary to determine whether any differences occurred during exercise.

Values were obtained for the entire range over which right atrial blood gases and pH were found to vary in the exercise tests performed in these studies. The close similarity between results from the two sampling sites indicates that it is admissible to sample from the right atrium for mixed venous blood during exercise in the Thoroughbred and hence such samples will be described as 'mixed venous' (denoted where necessary by the suffix  $v$ ) in these studies.

Figure 5.1: Pulmonary arterial and right atrial  $\text{Po}_2$  (mm Hg) during exercise in Thoroughbred horses.



**Figure 5.2: Pulmonary arterial and right atrial  $P_{CO_2}$  (mm Hg) during exercise in Thoroughbred horses.**

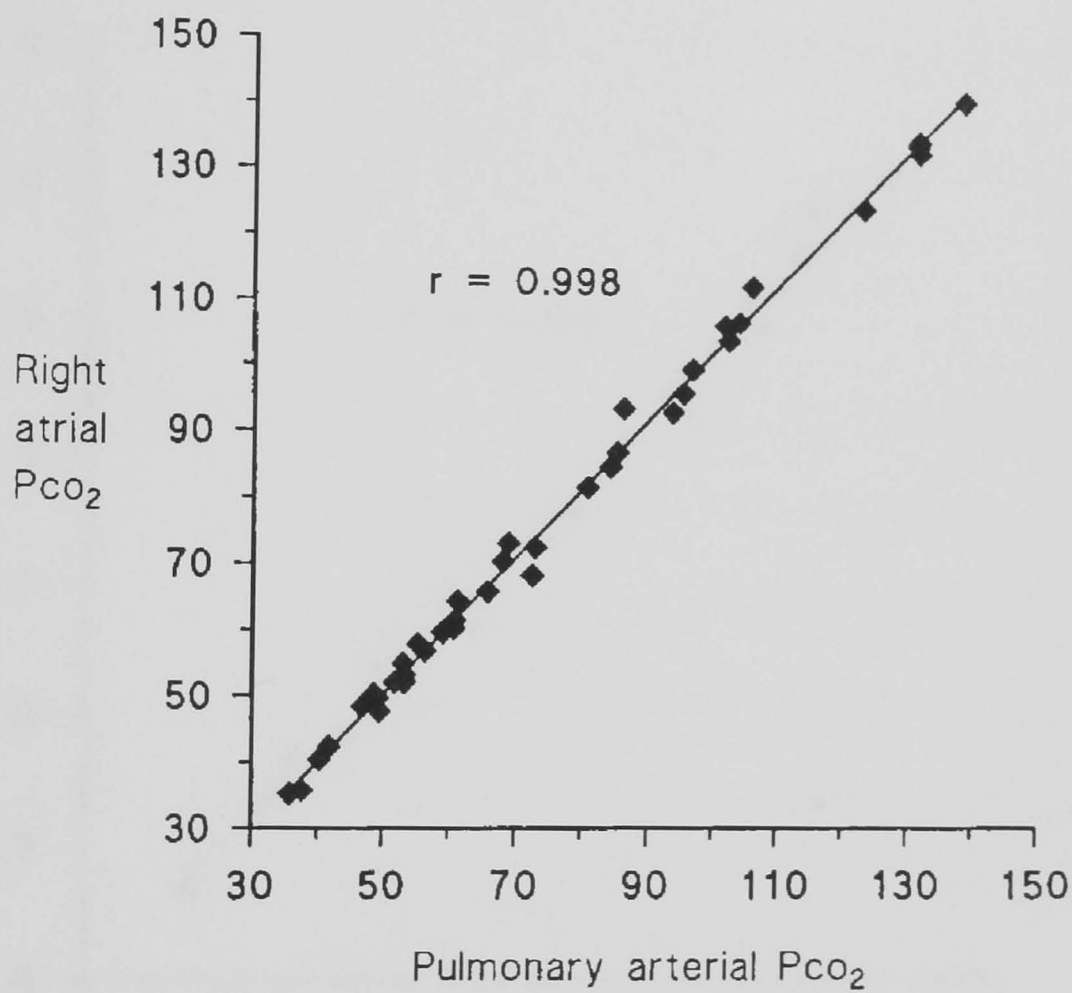
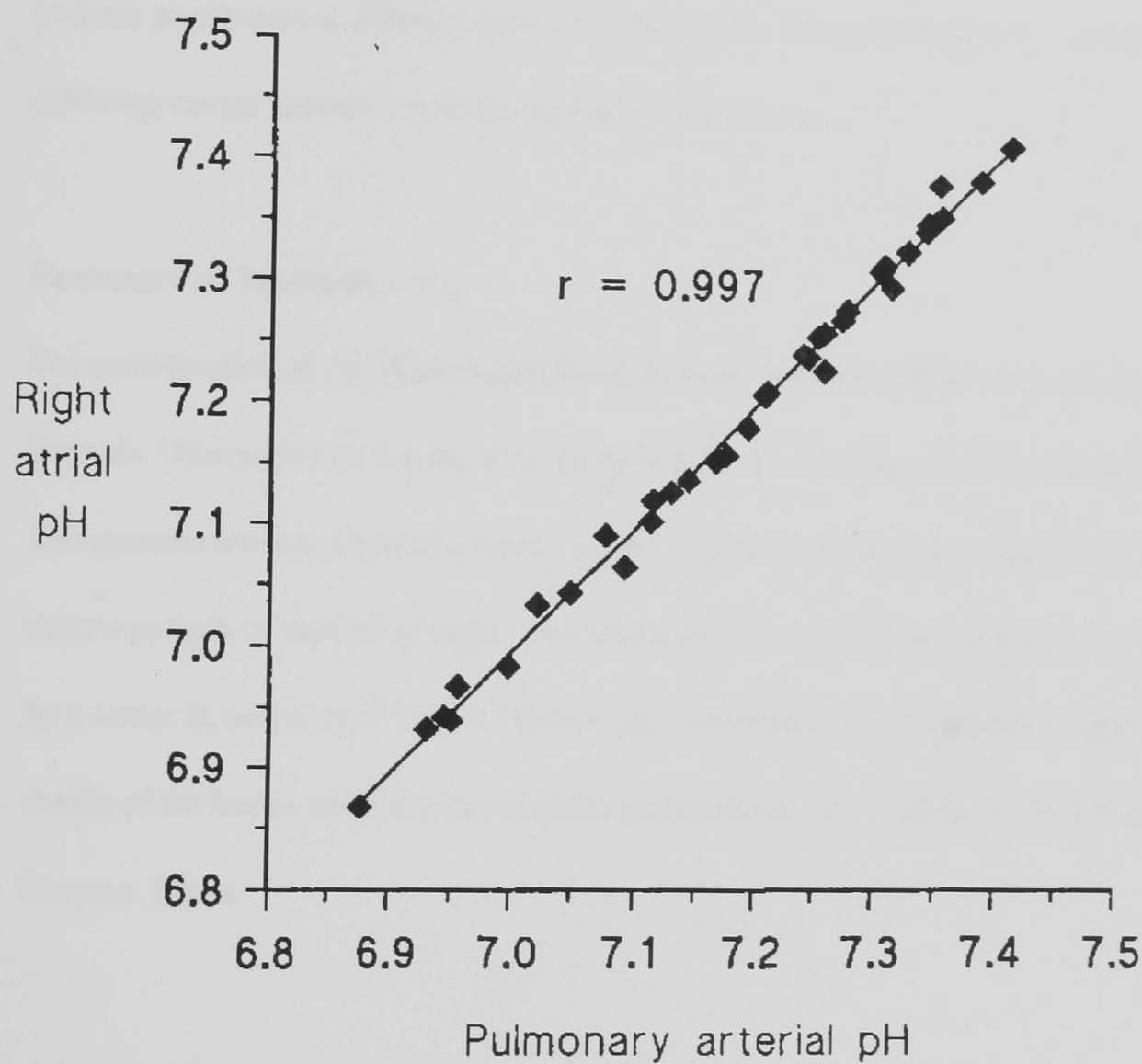


Figure 5.3: Pulmonary arterial and right atrial pH during exercise in Thoroughbred horses.



## CHAPTER SIX - THE KINETICS OF THE CHANGES IN RESPIRATION ASSOCIATED WITH THE ONSET OF CANTER/GALLOP IN NORMAL THOROUGHBRED HORSES

This chapter is concerned with the changes in respiratory parameters during the course of the two minute canter periods used in the exercise tests in this project. The changes in these parameters at different levels of exercise (i.e. between different gaits and between differing canter speeds) are discussed in the next chapter.

### Summary of Methods

For consideration of the kinetics of the respiratory response to fast exercise, data obtained from six Thoroughbred horses at the end of a fifteen week period of training were used. The exercise test consisted of a warm up period (at walk and trot) followed by four, two minute periods of canter<sup>1</sup> at eight, ten and twelve m.s<sup>-1</sup> with the treadmill level, followed by a canter at twelve m.s<sup>-1</sup> on a 3° incline; each separated by 10 minute walks. For further details of the horses used, the exercise test protocol and the method of data collection, see Chapter Three.

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<sup>1</sup>In discussion of the results of exercise tests, the term *canter* will be used to describe exercise at speeds of 6 m.s<sup>-1</sup> or greater. Although at 6, 8 and 10 m.s<sup>-1</sup>, Thoroughbred horses are normally cantering, by the time 12 m.s<sup>-1</sup> is achieved many horses are in fact galloping. The single term *canter* is however used for ease of style.

Synchronisation of respiration with locomotion occurs during both gaits (Attenburrow, 1982).

## Results

Data were collected from six normal Thoroughbred horses at ten second intervals during canters at 8, 10, 12 m.s<sup>-1</sup> and 12 m.s<sup>-1</sup> on a 3° incline.

In some cases measurements could not be obtained, for example the mass spectrometer signal for oxygen was lost during the 8 m.s<sup>-1</sup> canter for Briny and during all canters except 8 m.s<sup>-1</sup> for Flighty. Additionally, data for the first minute at 8 m.s<sup>-1</sup> of Buddy's test and 70 to 100 seconds of 12 m.s<sup>-1</sup> in Monty's test were lost. Furthermore, in a small number of files, it was not possible to match the flow and mass spectrometer signals, usually following the occurrence of abnormal breaths. As a result, the number of measurements at each time point is in some cases three to five, rather than six.

Since the Asyst computer programme involves a small delay (of approximately 250ms) between collection of each subfile, the time taken to collect twelve subfiles is approximately 123 seconds rather than 120. As a result, the final subfile sometimes overlapped into deceleration and was therefore ignored.

### *Individual Breath Characteristics*

Approximately 5,400 breaths were recorded during the various canter periods. The flow pattern of typical breaths for a cantering horse is shown in Figure 6.1. There is characteristically a rapid rise and fall in flow rate in both the inspiratory and expiratory phases. Inspiratory flow has a regular contour with a single or a slightly biphasic peak. In contrast, expiratory flow generally has a single peak and may have greater fluctuation in

contour, possibly due to the effect of footfall which occurs solely during expiration during canter.

The commonest pattern of flows was observed in 95.8% of breaths during canter. Two hundred and thirty four breaths were identified as varying from the common pattern (Figure 6.2). Ninety four abnormally small breaths were seen, the majority occurring in the acceleration phase of canter. They were seen in 4/6 horses during at least one canter. The number of small breaths per canter was 0-18 (median 1.5).

During acceleration to canter a period of 'breath-holding', varying in length from 0.6 to 8 seconds (median 1.85 seconds), was observed on ten occasions in nine individual canters. During these periods, there was for the most part no airflow, with occasional, intermittent small fluctuations of flow at low levels.

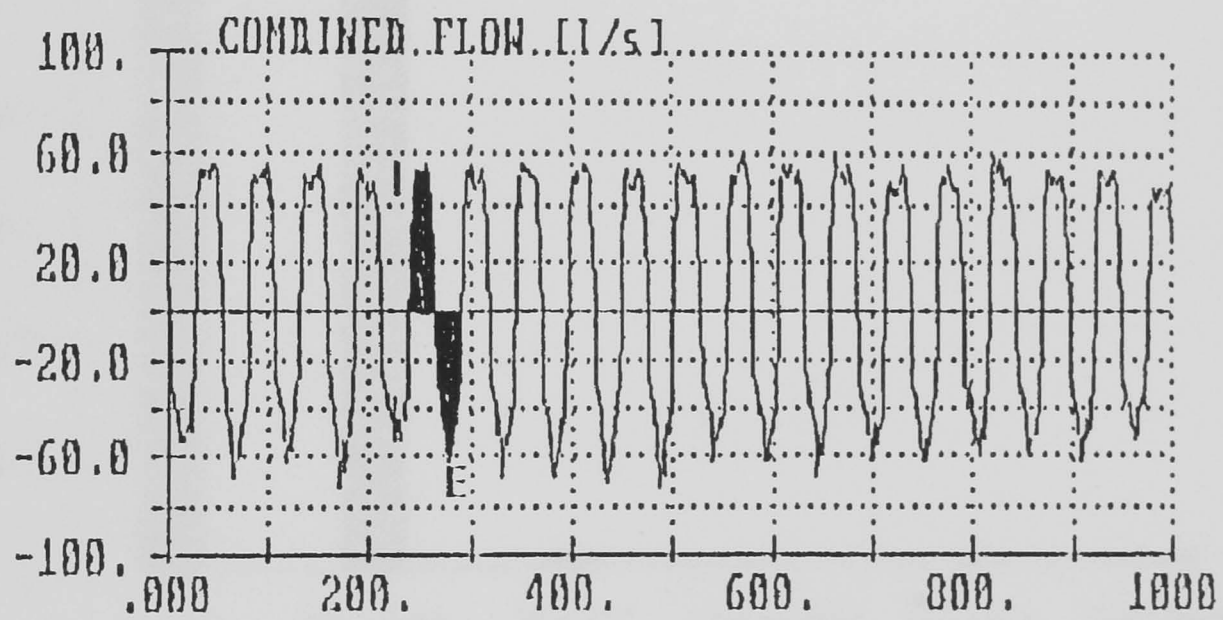
Ninety two other abnormal breaths were seen, the commonest form being one in which inspiration occurred in two more or less discrete parts followed by a single expiratory phase. These breaths (of which 48 were noted<sup>2</sup>) were referred to as 'double inspirations' and were 1.5-2 times the length of the normal breath.

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<sup>2</sup>This breath type was more common at higher workloads, its distribution being as follows:

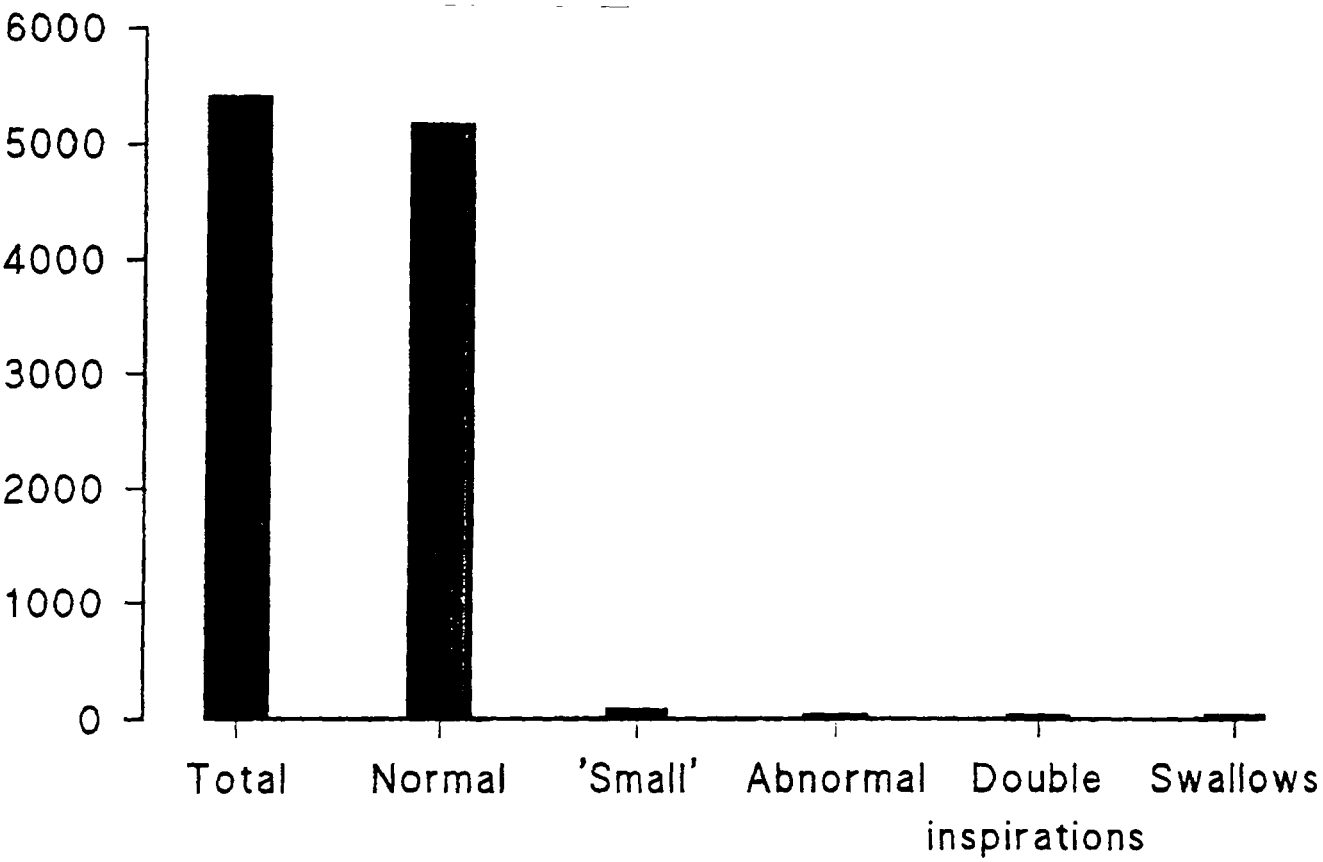
8 m.s <sup>-1</sup>	3 in 2/6 horses	Median 0
10 m.s <sup>-1</sup>	6 in 3/6 horses	Median 1
12 m.s <sup>-1</sup>	16 in 6/6 horses	Median 2.5
12 m.s <sup>-1</sup> @ 3°	23 in 4/6 horses	Median 4

Figure 6.1: Flow pattern for typical breaths from a cantering horse. Flow rate is plotted against time. Inspiratory flow is shown above the zero line and expiratory flow below. A single inspiration and expiration, marked 'I' and 'E' respectively are shaded (units: x axis: s. x 100; y axis: gas flow rate in  $\text{l.s}^{-1}$ ).





**Figure 6.2: Distribution of breath types during canter in normal Thoroughbred horses.**



Some of these breaths appeared to take about double the normal time for a breath and could, therefore, fit into the pattern of respiratory-locomotor synchrony without modification to stride pattern. The shorter of these breaths, however, would require some modification to breathing or stride pattern to allow maintenance of synchrony, either by altering slightly the time taken for the following breath or by a change in stride time for one stride. In the majority of cases the former explanation is the most likely as the horses appeared to show a very constant stride rate and did not often change leading leg on the treadmill during canter in these studies.

Other types of abnormal breaths occurred at least once in all horses except Monty, the number per canter ranging from 0-11 (median 0). The frequency of occurrence at the different work levels was:

8 m.s <sup>-1</sup>	23 in 4/6 horses
10 m.s <sup>-1</sup>	10 in 3/6 horses
12 m.s <sup>-1</sup>	2 in 1/6 horses
12 m.s <sup>-1</sup> @ 3°	9 in 3/6 horses

Periods of zero flow, referred to as 'flow hesitations' and thought initially to be associated with swallowing<sup>3</sup> were observed on 48 occasions during canter. They were seen in all

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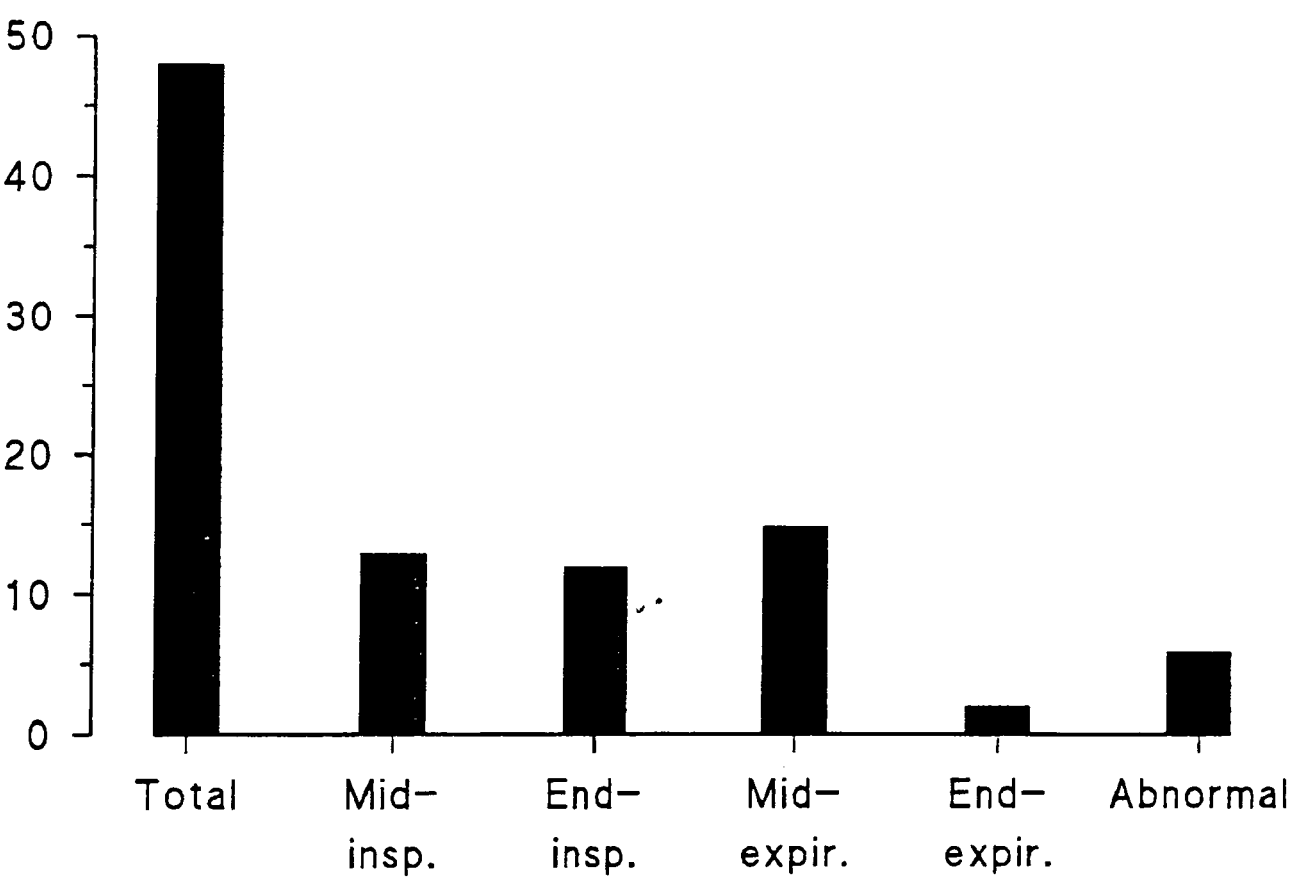
<sup>3</sup>This will be discussed further below.

horses and occurred between 0 and 10 times per canter (median 1). The frequency of occurrence at the different work rates was:

8 m.s <sup>-1</sup>	21 in 5/6 horses
10 m.s <sup>-1</sup>	9 in 5/6 horses
12 m.s <sup>-1</sup>	7 in 4/6 horses
12 m.s <sup>-1</sup> @ 3°	11 in 2/6 horses

The flow hesitation could occur at any stage of respiration, but was least likely to occur at the end of expiration (Figure 6.3). Occasionally flow hesitations occurred during a breath which was also abnormal in other ways.

**Figure 6.3: Distribution of flow hesitations according to phase of breath in which they occur.**



### *Kinetics of ventilatory parameters during canter*

The times taken to achieve a steady state during canter for the respiratory variables measured are shown in Tables 6.1-6.4. Graphic examples of the time course of respiratory variables are shown at the end of the Chapter (Figures 6.5 and 6.6). Tables 6.5-6.8 show the time at which individual horses reached a steady state for each variable.

#### *(i) Respiratory frequency*

With the onset of canter, respiratory frequency ( $f_b$ ) increased rapidly, reaching a plateau by 10 seconds at the three lower speeds and immediately<sup>4</sup> at 12 m.s<sup>-1</sup> at 3°.  $f_b$  then showed little variation except for occasions when frequency was reduced by the occurrence of a flow hesitation or double inspiration.

#### *(ii) Tidal Volume*

Tidal volume ( $V_t$ ) usually increased with the onset of canter in all animals, although one horse (Flighty), having a particularly high  $V_t$  whilst walking before canter, showed a slight decrease in  $V_t$  at 8 m.s<sup>-1</sup>.

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<sup>4</sup>As the first sub-file of a canter began at the point at which the horse reached the set canter speed, i.e. at the end of acceleration, 'immediately' is taken to mean following acceleration but at the start of the two minute period at the set canter speed. Acceleration took approximately four to five seconds at all speeds.

At the lowest canter speed,  $V_t$  was most variable, with two horses showing little change throughout the canter period and two others reaching a plateau at 10 and 40 seconds. By 60 seconds, Buddy (for whom no data were available for the first minute of canter) had already reached a steady state, whilst the final horse (Briny) showed most variation,  $V_t$  fluctuating between extreme values of 6.15l and 11.46l.

At the higher speeds, a more obvious plateau was detectable.  $V_t$  rose rapidly in the first 20 ( $10 \text{ m.s}^{-1}$ ) to 30 ( $12 \text{ m.s}^{-1}$  with and without incline) seconds, after which the rate of increase declined until a more or less steady state was maintained through the second minute.

At  $10 \text{ m.s}^{-1}$ , individual horses reached a plateau at 20, 30, 40, 50 and 70 seconds. The other animal appeared to plateau at 50 seconds before edging upwards to a higher plateau at 70 seconds. At  $12 \text{ m.s}^{-1}$ ,  $V_t$  levelled off at 20, 30, 40 and 60 (2 horses) seconds.

In the other horse (Monty), values for  $V_t$  were similar at 40 and 100 seconds, but the presence of a plateau could not be confirmed due to the lack of data between these points.

At  $12 \text{ m.s}^{-1}$  on a  $3^\circ$  incline, the plateaus were best defined and occurred at 40 (3 horses) 50 (2 horses) and 60 seconds.

At this speed one horse (Ross) showed a precipitous decline in  $V_t$  at 90 and 100 seconds, this was due to the occurrence of abnormally small breaths associated with flow hesitations at both sampling points.

**Table 6.1: Summary of times taken to reach steady state conditions for respiratory variables in Thoroughbred horses performing Standardised Exercise Tests**

*8 m.s<sup>-1</sup>*

<i>Time (s):</i>	0	10	20	30	40	50	60	70	80	90	100	110
$f_b$	0/6	6/6										
$V_t$	0/2	1/2	1/2	1/2	2/2		3/3					
$t_i$	0/6	6/6										
$t_e$	0/6	6/6										
$t_i/t_e$	0/6	6/6										
PIF	2/5	2/5	2/5	5/5			6/6					
PEF	3/5	4/5	4/5	4/5	5/5		6/6					
$\dot{V}O_2$	0/4	2/4	3/4	3/4	4/4		5/5					
$\dot{V}CO_2$	1/5	4/5	4/5	4/5	5/5		6/6					
$P_{ET}O_2$	0/4	0/4	3/4	3/4	4/4		5/5					
$P_EO_2$	0/4	0/4	1/4	1/4	4/4		4/5	4/5	5/5			
$P_{ET}CO_2$	0/6	6/6										
$P_ECO_2$	0/5	2/5	3/5	5/5			6/6					

Columns show number of horses at steady state as the numerator and number of horses for whom measurements were available as the denominator. Where the denominator is increased during a row, this is due to the addition of a horse for whom early data were not available.

**Table 6.2: Summary of times taken to reach steady state conditions for respiratory variables in Thoroughbred horses performing Standardised Exercise Tests**

*10 m.s<sup>-1</sup>*

<i>Time (s):</i>	0	10	20	30	40	50	60	70	80	90	100	110
$f_b$	0/6	6/6										
$V_t$	0/6	0/6	1/6	2/6	3/6	4/6	4/6	6/6				
$\dot{V}_e$	0/6	0/6	1/6	2/6	3/6	4/6	4/6	6/6				
$t_i$	0/6	6/6										
$t_e$	0/6	5/6	5/6	6/6								
$t_i/t_e$	0/6	6/6										
PIF	0/6	0/6	1/6	3/6	3/6	4/6	5/6	5/6	5/6	6/6		
PEF	0/6	1/6	2/6	3/6	5/6	6/6						
$\dot{V}O_2$	0/5	1/5	1/5	3/5	4/5	4/5	4/5	4/5	5/5			
$\dot{V}CO_2$	0/6	1/6	1/6	1/6	2/6	3/6	3/6	4/6	6/6			
$P_{ET}O_2$	0/5	0/5	4/5	5/5								
$P_EO_2$	0/5	2/5	5/5									
$P_{ET}CO_2$	0/6	0/6	6/6									
$P_ECO_2$	0/6	2/6	4/6	4/6	5/6	6/6						

Columns show number of horses at steady state as the numerator and number of horses for whom measurements were available as the denominator.



**Table 6.3: Summary of times taken to reach steady state conditions for respiratory variables in Thoroughbred horses performing Standardised Exercise Tests**

12 m.s<sup>-1</sup>

Time (s):	0	10	20	30	40	50	60	70	80	90	100	110
f <sub>b</sub>	0/6	6/6										
V <sub>t</sub>	0/5	0/5	1/5	2/5	3/5	3/5	5/5					
Ṡ <sub>e</sub>	0/5	0/5	1/5	1/5	2/5	2/5	3/5	5/5				
t <sub>i</sub>	0/6	6/6										
t <sub>e</sub>	0/6	5/6	6/6									
t <sub>i</sub> /t <sub>e</sub>	0/6	6/6										
PIF	0/5	0/5	1/5	1/5	2/5	2/5	4/5	5/5				
PEF	0/5	0/5	1/5	2/5	2/5	2/5	4/5	4/5	5/5*			
Ṡ <sub>o2</sub>	0/4	0/4	1/4	1/4	1/4	2/4	2/4	2/4	2/4	4/4¶		
Ṡ <sub>co2</sub>	0/5	0/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	4/5	4/5	4/5
P <sub>ET</sub> O <sub>2</sub>	0/5	0/5	4/5	5/5								
P <sub>E</sub> O <sub>2</sub>	0/5	1/5	2/5	3/5	5/5							
P <sub>ET</sub> co <sub>2</sub>	0/5	0/5	1/5	1/5	1/5	2/5	4/5	4/5	4/5	5/5		
P <sub>E</sub> co <sub>2</sub>	0/5	0/5	1/5	1/5	1/5	2/5	4/5	4/5	4/5	5/5		

Columns show number of horses at steady state as the numerator and number of horses for whom measurements were available as the denominator.

\* final horse (80s) showed trend to increase throughout C12  
 ¶ peak values at 90s in two horses which tended to level out at 70-80s

**Table 6.4: Summary of times taken to reach steady state conditions for respiratory variables in Thoroughbred horses performing Standardised Exercise Tests**

*12 m.s<sup>-1</sup> @ 3 °*

<i>Time (s):</i>	0	10	20	30	40	50	60	70	80	90	100	110
$f_b$	6/6											
$V_t$	0/6	0/6	0/6	0/6	3/6	5/6	6/6					
$\dot{V}_e$	0/6	0/6	0/6	1/6	3/6	5/6	6/6					
$t_i$	0/6	6/6										
$t_e$	4/6	6/6										
$t_i/t_e$	0/6	6/6										
PIF	0/6	0/6	2/6	2/6	3/6	5/6	6/6					
PEF	0/5	0/5	4/5	4/5	4/5	4/5	5/5					
$\dot{V}_{O_2}$	0/3	0/3	0/3	0/3	2/3	3/3	4/4					
$\dot{V}_{CO_2}$	0/5	0/5	0/5	0/5	1/5	1/5	1/5	1/5	6/5			
$P_{ET}O_2$	0/6	6/6										
$P_EO_2$	0/5	3/5	4/5	4/5	4/5	5/5						
$P_{ET}CO_2$	0/5	0/5	0/5	0/5	0/5	0/5	2/5	3/5	5/5			
$P_ECO_2$	0/4	0/4	0/4	0/4	0/4	1/4	3/5	4/5	4/5	4/5	4/5	4/5

Columns show number of horses at steady state as the numerator and number of horses for whom measurements were available as the denominator.

**Table 6.5: Summary of times taken by individual horses to reach steady-state conditions for respiratory variables during standardised exercise tests**

8 m.s <sup>-1</sup>												
<i>Time (s):</i>	0	10	20	30	40	50	60	70	80	90	100	110
f <sub>b</sub>		All										
V <sub>t</sub>		F			G		Bu					
t <sub>i</sub>		All										
t <sub>e</sub>		All										
t <sub>i</sub> /t <sub>e</sub>		All										
PIF	FR			BGM			Bu					
PEF	DFR	G			M		Bu					
Ṡo <sub>2</sub>		GR	F		M		Bu					
Ṡco <sub>2</sub>	F	BGR			M		Bu					
P <sub>ET</sub> O <sub>2</sub>			GMR		F		Bu					
P <sub>E</sub> O <sub>2</sub>			R		FGM				Bu			
P <sub>ET</sub> co <sub>2</sub>		All										
P <sub>E</sub> co <sub>2</sub>		GR	F	BM			Bu					
Key to horses in Tables 6.5 - 6.8		B	=	Briny		F	=	Flighty		M	=	Monty
		Bu	=	Buddy		G	=	Goldie		R	=	Ross

**Table 6.6: Summary of times taken to reach steady-state conditions for respiratory variables in Thoroughbred horses performing standardised exercise tests**

	10 m.s <sup>-1</sup>											
<i>Time (s):</i>	0	10	20	30	40	50	60	70	80	90	100	110
f <sub>b</sub>		All										
V <sub>t</sub>			B	M	R	F		BuG				
$\dot{V}_e$			B	M	R	F		BuG				
t <sub>i</sub>		All										
t <sub>e</sub>		BFGMR		Bu								
t <sub>i</sub> /t <sub>e</sub>		All										
PIF			B	FG		M	R			Bu		
PEF		B	M	G	BuR	F						
$\dot{V}_{O_2}$		B		GM	R				Bu			
$\dot{V}_{CO_2}$		B			R	M		G	BuF			
P <sub>ET</sub> O <sub>2</sub>			BGM	Bu								
P <sub>E</sub> O <sub>2</sub>		BR	BuGM									
P <sub>ET</sub> CO <sub>2</sub>			All									
P <sub>E</sub> CO <sub>2</sub>		BM	BuR		F	G						

**Table 6.7: Summary of times taken to reach steady-state conditions for respiratory variables in Thoroughbred horses performing standardised exercise tests**

12 m.s <sup>-1</sup>												
<i>Time (s):</i>	0	10	20	30	40	50	60	70	80	90	100	110
f <sub>b</sub>		All										
V <sub>t</sub>			B	F	G		BuG					
$\dot{V}_e$			B		R		Bu	FG				
t <sub>i</sub>		All										
t <sub>e</sub>		BuFGMR	B									
t <sub>i</sub> /t <sub>e</sub>		All										
PIF			B		F		BuR	G				
PEF			B	R			BuG		F			
$\dot{V}_{O_2}$			B			Bu				GR		
$\dot{V}_{CO_2}$			B							BuFG		
P <sub>ET</sub> O <sub>2</sub>			BBuGM	R								
P <sub>E</sub> O <sub>2</sub>		R	B	Bu	GM							
P <sub>ET</sub> CO <sub>2</sub>			B			Bu	FG			R		
P <sub>E</sub> CO <sub>2</sub>			B			Bu	FG			R		

**Table 6.8: Summary of times taken to reach steady-state conditions for respiratory variables in Thoroughbred horses performing standardised exercise tests**

12 m.s<sup>-1</sup> @ 3°

<i>Time (s):</i>	0	10	20	30	40	50	60	70	80	90	100	110
f <sub>b</sub>	All											
V <sub>t</sub>					BMRG	BuF	G					
$\dot{V}_e$				B	MR	BuF	G					
t <sub>i</sub>		All										
t <sub>e</sub>	BuGMR	BF										
t <sub>i</sub> /t <sub>e</sub>		All										
PIF			BuF		R	BG	M					
PEF			BBuFR				G					
$\dot{V}_{O_2}$					BR	Bu	G					
$\dot{V}_{CO_2}$					Bu				BFGR			
P <sub>ET<sup>O</sup>2</sub>		All										
P <sub>E<sup>O</sup>2</sub>		BBuR	G			M						
P <sub>ET<sup>CO</sup>2</sub>							FG	B	BuM			
P <sub>E<sup>CO</sup>2</sub>						Bu	BG	F				

### *(iii) Minute Volume*

Reflecting the variability of  $V_t$  at  $8 \text{ m.s}^{-1}$ , minute volume ( $\dot{V}_e$ ) at this speed was also somewhat variable, showing a plateau in only three horses, although all horses showed an increase in  $\dot{V}_e$ .

Again reflecting  $V_t$ , at the higher speeds  $\dot{V}_e$  increased substantially in the first 20 to 30 seconds of canter and showed a more or less steady state through the second minute. At  $10 \text{ m.s}^{-1}$ , individuals reached plateaus by 20, 30, 40 and 50 seconds, with the remaining two horses levelling out at 70 seconds.

At  $12 \text{ m.s}^{-1}$ , plateaus were seen at 20, 40, 60 and 70 (two horses) seconds. As for  $V_t$ , values for  $\dot{V}_e$  for Monty were similar at 40 and 100 seconds, but the presence of a plateau could not be confirmed due to the lack of data between these points. At  $12 \text{ m.s}^{-1} @ 3^\circ$ , plateaus for  $\dot{V}_e$  were least variable and were attained at 30, 40 (2 horses), 50 (2 horses) and 60 seconds.

### *(iv) Inspiratory and expiratory times*

Inspiratory time ( $t_i$ ) and expiratory time ( $t_e$ ) both fell with the onset of canter and the increase in respiratory rate. After 10 seconds of canter,  $t_i$  showed very little change, both for mean values and in individual horses, except for a few occasions when the occurrence of single abnormal inspirations in a sample prolonged it slightly. These abnormal breaths did not appear to result in loss of respiratory-locomotory synchrony, certainly not in terms of synchronisation of rate.

$t_e$  varied little after 10 seconds at  $8 \text{ m.s}^{-1}$  in five horses but in one animal (Ross),  $t_e$  was prolonged on four occasions due to the occurrence of swallows in expiration. At  $10 \text{ m.s}^{-1}$ ,  $t_e$  attained a plateau by 10 seconds in five horses and by 30 seconds in the other. At  $12 \text{ m.s}^{-1}$ , a plateau was reached at 10 seconds in five horses and in 20 seconds in the other, whilst at  $12 \text{ m.s}^{-1} @ 3^\circ$ ,  $t_e$  reached steady state immediately in four horses and 10 seconds in two.

At all speeds,  $t_i$  and  $t_e$  showed occasional variations from the steady state when flow hesitations or other individual abnormal breaths occurred in a sampled group.

$t_i/t_e$  remained fairly constant after the first 10 seconds of canter, with variations occurring with those in  $t_i$  or  $t_e$  already described. The ratio was more variable at  $8 \text{ m.s}^{-1}$  than at the higher speeds.

#### *(v) Peak respiratory flow rates*

Peak respiratory flow rates increased with the onset of canter, reaching a plateau by 20 to 30 seconds and showing little change after this. In individual horses, the pattern was similar.

At  $8 \text{ m.s}^{-1}$ , PIF reached a plateau immediately in two horses and by 30 seconds in three horses. In the remaining horse, for whom no canter data were available in the first minute, a plateau had been reached by the start of the second minute.

At  $10 \text{ m.s}^{-1}$ , PIF reached a plateau at 30 seconds in two horses and in other horses at 20,



50, 60 and 90 seconds. At  $12 \text{ m.s}^{-1}$ , individual plateaus for PIF were reached at 20, 40, 60 (two horses) and 70 seconds. In the remaining horse (Monty) PIF appeared to plateau at 40 seconds but as data were lost between 60 and 90 seconds this could not be confirmed. At  $12 \text{ m.s}^{-1}$  @  $3^\circ$ , two horses reached a plateau for PIF at 20 seconds and the others at 40, 50 (2 horses) and 60 seconds.

At  $8 \text{ m.s}^{-1}$ , peak expiratory flow rate (PEF) was most variable, with three horses showing marked variations around the plateau level of PIF. At this speed, plateaus were reached immediately in three horses and by 10 and 40 seconds in the other two. The final horse (no data available in the first minute) had reached a steady state by the end of the first minute.

At  $10 \text{ m.s}^{-1}$ , individual plateaus were attained for PEF at 10, 20, 30, 40 (two horses) and 50 seconds, although two horses, Goldie and Buddy, showed an upward drift in the second minute to a higher plateau during the last 30 seconds of canter. Goldie showed a similar pattern at the two highest speeds, appearing to reach a plateau at 30 seconds before PEF increased again and finally plateaued at 60 seconds at each speed.

Plateaus were reached for PEF at 20, 30 and 60 seconds (two horses) at  $12 \text{ m.s}^{-1}$ . In one horse (Monty) for whom data was lost between 60 and 90 seconds a plateau could not be identified, but appeared to be close at 40 seconds. The sixth horse (Flighty) showed a trend for increasing PEF throughout canter at this speed, although a plateau may have been reached at 80 seconds.

At the highest speed ( $12\text{m.s}^{-1}$  @  $3^\circ$ ), four horses reached a plateau for PEF at 20 seconds and another at 60 seconds. Monty appeared to approach a plateau at 40 seconds but this could not be confirmed as after 70 seconds of canter, the flow signal from the right nostril inverted at peak flow rates (i.e. direction of recorded flow reversed towards inspiration during the peak expiratory flow period - this was identified as artefact as the left nostril flow remained expiratory during this time).

*(vi) Oxygen uptake and carbon dioxide production*

As explained above, data were available for  $\dot{V}\text{O}_2$  for only five animals, with data from Briny at  $8\text{ m.s}^{-1}$  and Flighty at the other speeds, missing.

Mean oxygen uptake ( $\dot{V}\text{O}_2$ ), standardised for body weight, reached more or less a steady state by 30 seconds at 8, 10 and  $12\text{ m.s}^{-1}$ . At  $12\text{ m.s}^{-1}$  @  $3^\circ$  a plateau was achieved at 50 seconds.

For individual horses, at  $8\text{ m.s}^{-1}$ , a plateau was reached at 10 (2 horses), 20 and 40 seconds for the other horse, for whom data were missing for the first minute, values were within the range of the plateau values of the other horses for the second minute. At  $10\text{ m.s}^{-1}$ , a plateau was reached at 10, 30 (2 horses), 40 and 80 seconds.

At  $12\text{ m.s}^{-1}$ , there were insufficient data points to define a plateau in one horse and in two others,  $\dot{V}\text{O}_2$  tended to rise continuously until showing signs of levelling off at 70-80 seconds, with peak values at 90 seconds. In the other two horses,  $\dot{V}\text{O}_2$  plateaued at 20 and 50 seconds. At the highest speed,  $\dot{V}\text{O}_2$  levelled off at 40 seconds in two horses and 50

seconds in one. In the remaining two horses, there were insufficient data points to identify a plateau although one horse (Goldie - for whom data was missing from 30-50 seconds) appeared to have plateaued by 60 seconds.

The highest individual values for  $\dot{V}O_2$  for each individual horse were as follows

Briny	114.9 ml.min. <sup>-1</sup> kg <sup>-1</sup>
Goldie	187.2 ml.min. <sup>-1</sup> kg <sup>-1</sup>
Flighty	( $\dot{V}O_2$ not determined at >8 m.s <sup>-1</sup> )
Monty	130.3 ml.min. <sup>-1</sup> kg <sup>-1</sup> <sup>5</sup>
Ross	150.2 ml.min. <sup>-1</sup> kg <sup>-1</sup>
Buddy	167.0 ml.min. <sup>-1</sup> kg <sup>-1</sup>

The mean of the highest  $\dot{V}O_2$  values for individual horses was 149.9 (±11.5) ml.min.<sup>-1</sup>kg<sup>-1</sup>.

Mean  $\dot{V}CO_2$  also rose with the onset of canter, plateaus were reached at:

- 30 seconds at 8 m.s<sup>-1</sup>,
- 40 seconds at 10 m.s<sup>-1</sup>,
- 70 seconds at 12 m.s<sup>-1</sup>, and
- 60 seconds at 12 m.s<sup>-1</sup> @ 3°.

At 8 m.s<sup>-1</sup> individual horses reached plateaus for  $\dot{V}CO_2$  immediately (one horse) and at 10 (3 horses) and 40 seconds.

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<sup>5</sup>n.b. no data points after 70 seconds for 12 m.s<sup>-1</sup> @ 3°

The other horse (no data for minute one) had plateaued by 60 seconds.

At  $10 \text{ m.s}^{-1}$ , individual animals reached a plateau at 10, 40, 50, 70 and 80 seconds, with the final horse showing no clear plateau but appearing to be approaching a steady state at 80 seconds although the highest value for this horse occurred at 110 seconds.

$\dot{V}\text{CO}_2$  at  $12 \text{ m.s}^{-1}$  reached a steady state at 20 seconds in one horse, but plateaus were poorly defined in the remainder. In three cases,  $\dot{V}\text{CO}_2$  seemed to have levelled off by 70-90 seconds, but in another it continued to rise throughout exercise. There were insufficient data points to draw conclusions regarding the other horse. Plateaus were also hard to define at  $12 \text{ m.s}^{-1}$  @  $3^\circ$ , but seemed to occur at 40 seconds in one horse and 80 seconds in another. The remainder seemed to level out between 50 and 80 seconds.

*(vii) Expired gas concentrations*

As for  $\dot{V}\text{O}_2$ , data were obtained from only five horses at each speed for expired  $\text{Po}_2$  tensions.

End-tidal  $\text{Po}_2$  ( $\text{P}_{\text{ET}}\text{O}_2$ ) fell with the onset of canter, mean values changing little after 10 seconds of canter. At  $8 \text{ m.s}^{-1}$ , three horses reached a steady state by 20 seconds and a fourth by 40 seconds. The other horse, for whom data was only available for the second minute at this speed had reached a steady state by that time.

At  $10 \text{ m.s}^{-1}$ , four horses had reached steady state by 20 seconds and one by 30. The picture was similar at  $12 \text{ m.s}^{-1}$ , although the last horse (Ross) to reach a plateau remained

more variable. At  $12 \text{ m.s}^{-1}$  on the incline, all horses reached a steady state for  $P_{\text{ET}}\text{O}_2$  by 10 seconds of canter.

Mixed expired  $\text{Po}_2$  ( $P_{\text{E}}\text{O}_2$ ) fell during canter and at  $8 \text{ m.s}^{-1}$  was near to steady state by 20 seconds in one horse, and by 40 seconds. The fifth horse, for which data was unavailable in the first minute appeared to level out at 80 seconds.

$P_{\text{E}}\text{O}_2$  levelled out at 10 (two animals) and 20 seconds (three horses) at  $10 \text{ m.s}^{-1}$ , whilst individual horses attained a steady state at  $12 \text{ m.s}^{-1}$  at 10, 20, 30 and 40 seconds. Monty, for whom few data points were available had probably reached steady state by 40 seconds at the latter speed. At  $12 \text{ m.s}^{-1}$  @  $3^\circ$ , three horses had reached a plateau for this parameter at 10 seconds and a fourth at 20 seconds. Although, again, several points were missing for Monty, he showed little change in ( $P_{\text{E}}\text{O}_2$ ) after 50 seconds of canter.

End-tidal  $\text{Pco}_2$  ( $P_{\text{ET}}\text{CO}_2$ ) rose as speed increased and a plateau was reached for mean values:

at 10 seconds at  $8 \text{ m.s}^{-1}$

at 20 seconds at  $10 \text{ m.s}^{-1}$

at 60 seconds at  $12 \text{ m.s}^{-1}$  and

at 70 seconds at  $12 \text{ m.s}^{-1}$  @  $3^\circ$

Individual horses followed this pattern closely at the two lower speeds (with one horse showing a trend to keep increasing  $P_{\text{ET}}\text{CO}_2$  at  $10 \text{ m.s}^{-1}$ ). At  $12 \text{ m.s}^{-1}$ , there was more individual variation within and between horses, with the rise in  $P_{\text{ET}}\text{CO}_2$  levelling off at 20,

50, 60 (2 horses) and 90 seconds for individual horses (data unavailable for 60 to 90 seconds in one horse, precluding identification of a plateau). At 12 m.s<sup>-1</sup> on the incline the rise in P<sub>ET</sub>CO<sub>2</sub> was greatest, plateaus were reached at 60 (2 horses), 70 and 80 (2 horses) seconds.

Data were unavailable after 70 seconds in the sixth horse, at which point P<sub>ET</sub>CO<sub>2</sub> was the highest of any horse at that point, but a plateau had not occurred.

For mixed expired Pco<sub>2</sub> (P<sub>E</sub>CO<sub>2</sub>) at 8 m.s<sup>-1</sup>, plateaus were seen at 10 seconds (two horses), 20 seconds and 30 seconds (two horses), whilst showing little change in the second minute for the horse for whom data was missing for the first minute.

At 10 m.s<sup>-1</sup>, steady state was reached at 10 (2 horses), 20 (2 horses), 40 and 50 seconds.

At 12 m.s<sup>-1</sup>, individuals reached plateau for P<sub>E</sub>CO<sub>2</sub> at 20, 50, 60 (2 horses) and 90 seconds. There were insufficient data points to discern a plateau in the other animal.

At the highest workload, plateaus were discernible at 50, 60 and 70 seconds, whilst a fourth horse (missing data for 30-50 seconds) appeared to have levelled out by 60 seconds. In one horse, a peak occurred at 50 seconds, with no data after 70 seconds, preventing further assessment and in the other animal (no values for 10, 20, 40 and 80 seconds) P<sub>E</sub>CO<sub>2</sub> continued to rise through canter.

#### *(viii) Mixed venous blood temperature*

Mixed venous blood temperature rose steadily throughout the canter period at all speeds.

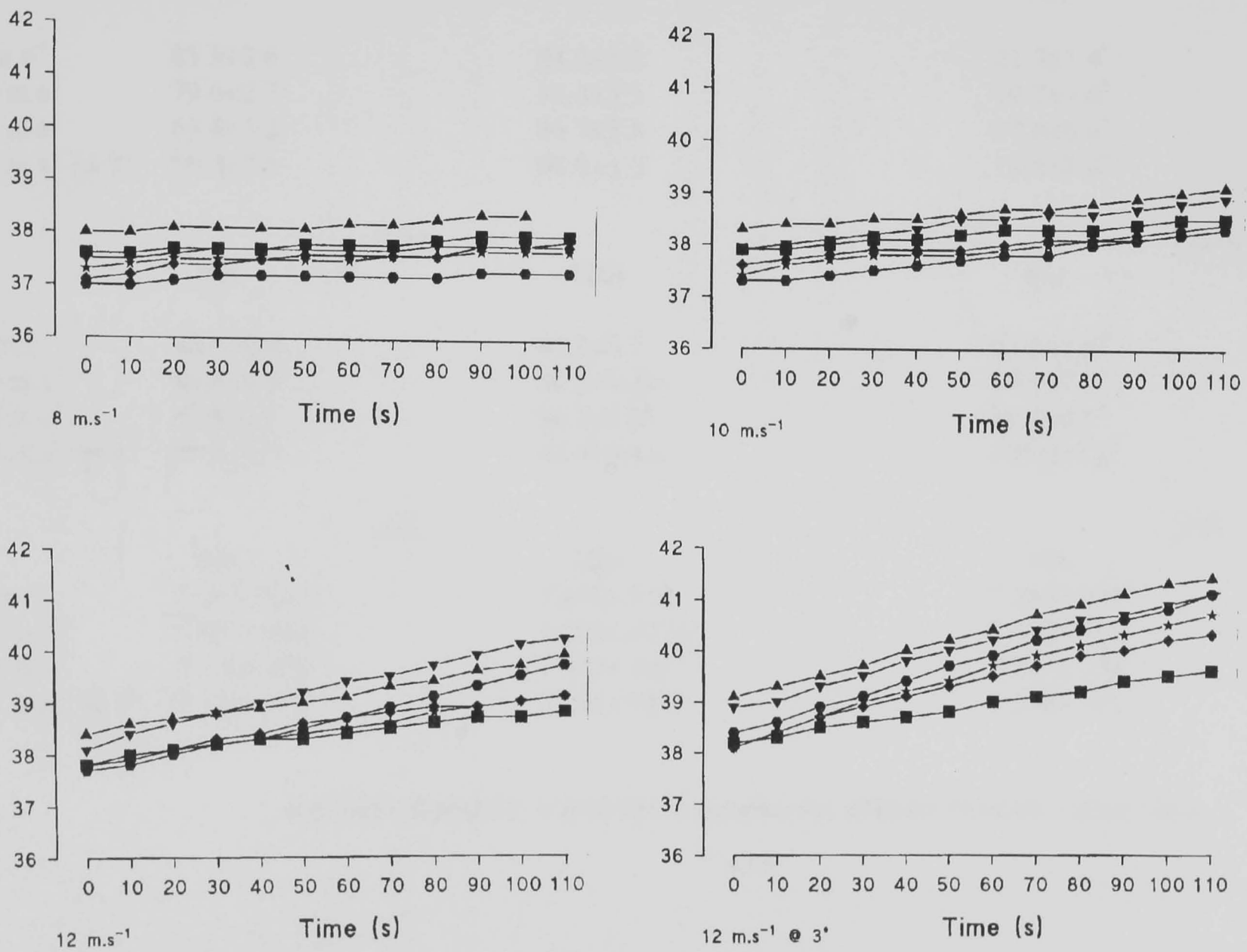
The rate of increase in temperature through the canter period increased with increasing work load (Figure 6.4).

*(ix) Blood gases*

Arterial and mixed venous blood samples were drawn at the end of each minute of exercise for blood gas analysis. Data for  $P_{O_2}$ ,  $P_{CO_2}$  and pH, corrected for body temperature, are shown in Table 6.9. Canter exercise was associated with the development of hypoxaemia, hypercapnia and acidaemia as described previously (see for example, Bayly *et al.*, 1983; Butler *et al.*, 1993a). The changes in these variables associated with differing levels of exercise are described in the next chapter.

A comparison of data collected at the end of the first and second minutes of each canter revealed no significant changes in  $P_{aO_2}$  or  $P_{\bar{v}O_2}$  at any speed. In contrast,  $P_{CO_2}$  was significantly higher and blood pH significantly lower at the end of the second minute at all canter speeds except 8 m.s<sup>-1</sup> for arterial blood and at all canter speeds for mixed venous blood.

Figure 6.4: Changes in mixed venous blood temperature ( $^{\circ}\text{C}$ ) with time for Thoroughbred horses during two minute canters on a treadmill.





**Table 6.9: Blood gas values for six Thoroughbred horses during canter**

	$P_aO_2$		$P_{\bar{v}}O_2$	
	60s	120s	60s	120s
8 m.s <sup>-1</sup>	85.5±2.6	84.3±1.1	21.7±1.4*	21.3±1.0
10 m.s <sup>-1</sup>	79.6±2.7	76.3±3.5	19.7±1.0*	19.3±0.8
12 m.s <sup>-1</sup>	69.8±3.2	66.7±2.8	17.6±0.6*	17.5±1.2
12 m.s <sup>-1</sup> @ 3°	59.0±2.5	60.2±3.0	13.5±1.0*	12.6±1.5*
	$P_aCO_2$		$P_{\bar{v}}CO_2$	
	60s	120s	60s	120s
8 m.s <sup>-1</sup>	44.9±0.8	47.1±0.7	63.0±1.6*	67.4±1.5 $\alpha$
10 m.s <sup>-1</sup>	46.5±0.9	50.1±1.3 $\alpha$	69.1±3.5*	79.0±4.5 $\alpha$
12 m.s <sup>-1</sup>	50.6±1.6	56.5±2.2 <sup>B</sup>	80.3±6.0*	103.6±9.4 $\alpha$
12 m.s <sup>-1</sup> @ 3°	56.6±1.9	63.4±3.4 $\alpha$	103.0±5.6*	137.1±9.3 <sup>*B</sup>
	$pH_a$		$pH_{\bar{v}}$	
	60s	120s	60s	120s
8 m.s <sup>-1</sup>	7.451±.016	7.442±.014	7.383±.017*	7.371±.015 $\alpha$
10 m.s <sup>-1</sup>	7.435±.021	7.404±.027 $\alpha$	7.358±.027*	7.316±.030 $\alpha$
12 m.s <sup>-1</sup>	7.376±.031	7.302±.045 <sup>B</sup>	7.297±.038*	7.189±.052 $\alpha$
12 m.s <sup>-1</sup> @ 3°	7.156±.048	7.130±.052 <sup>B</sup>	7.238±.059*	7.008±.060 <sup>*B</sup>

$\alpha$   $p<0.05$ ;  $\beta$   $p<0.01$ ;  $\gamma$   $p<0.001$  = significantly different from 60 second value

\* n=5

## Discussion

### *Individual breath characteristics*

Whilst during the warm up period of walk and trot there was considerable breath-to-breath variation in terms of both flow pattern and volume, with the onset of canter and respiratory-locomotory synchrony, a readily identifiable 'normal' breath form was seen and the majority of breaths conformed to this pattern. The commonest departure from the normal breath was the abnormally small breath which was seen almost invariably during the early, acceleration phase of canter.

Although the majority of breaths followed the normal pattern, it is apparent that horses with no sign of abnormal respiratory function occasionally take 'abnormal' breaths, i.e. breaths of an unusual pattern, during canter. Equally, all but one of the horses had periods of zero flow during canter and immediately resumed breathing in synchrony with stride.

Flow hesitations were at first thought to be due to swallowing, but were later observed in a referred two year old Thoroughbred undergoing exercise videoendoscopy with simultaneous recording of respiratory flow rates. In this horse, flow hesitations occurred in the presence of an open glottis indicating that they may occur independently of swallowing.

The number of abnormal breaths and flow hesitations tended to reduce as worklevel increased. This may reflect a reduced tendency by the horse to override the normal pattern of breathing in response to extraneous factors as the demands of exercise are increased. An exception to this were the breaths in which inspiration was markedly biphasic as these

breaths occurred nearly eight times as frequently during the highest speed canter than at  $8 \text{ m}\cdot\text{s}^{-1}$ .

Although these 'double inspiratory' breaths, having a higher tidal volume, would also have a higher  $V_A$ , they cannot represent a method for increasing  $\dot{V}_A$  during high speeds as the breath duration is increased compared to the normal breath, thus offsetting the advantage of the reduction in  $V_d/V_t$  during these breaths. Additionally, they occur so infrequently (approximately two per minute on average, the maximum frequency seen being four per minute) as to have negligible effect on overall  $\dot{V}_A$ . Recently Jolly, Art and Lekeux (1994) have also reported the occurrence of 'double inspirations', calling such breaths 'big respiratory cycles'. These workers performed individual breath analysis of the breaths following the double inspiratory breaths and reported that for a few breaths after a double inspiration, oxygen extraction was improved. They concluded that the double inspiratory breath increases functional residual capacity and improves oxygen extraction and carbon dioxide excretion.

#### *Changes in respiratory variables with time during canter exercise*

The kinetics of the respiratory response were examined in order to determine when a steady state was likely to occur during cantering at the work levels used in these tests, so that data from a steady state situation could be analysed in the following studies. Since the final subfile of a series collected during a canter may include an odd, unrepresentative abnormal breath, if a steady state has been reached early in the canter, then an earlier subfile could validly be used for comparison with reference data.

This would be useful in a clinical setting, where it may not be possible to repeat test a horse. In such a situation, if only the last few seconds of canter could be used for assessing respiration, then a single, unrepresentative breath at this time could distort the test findings, but if an earlier subfile could validly be used, the test would still be interpretable.

Since horses synchronise respiratory rate and stride rate during canter it is unsurprising that  $f_b$ ,  $t_i$  and  $t_e$  reach a steady state very quickly at constant speed, - within 10 seconds in all but two cases. Although the major increase in both  $V_i$  and  $\dot{V}_e$  occurred in the first 20-30 seconds of canter, these variables generally took longer to reach a plateau than  $f_b$ , but in all cases a steady state was reached by 70 seconds of canter at the latest.

PIF and PEF had generally reached steady state by the end of the first minute (in 21/23 canters for PIF and 21/22 for PEF). Although on one occasion (Flighty at 12 m.s<sup>-1</sup>) PEF may not have truly reached a plateau, from 80 seconds onwards there was relatively little change in its value (83.4-86.9 l.s<sup>-1</sup>).

Both  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$  showed a trend towards longer times to steady state with increasing speed up to 12 m.s<sup>-1</sup>, but in all cases except one reached a plateau by 90 seconds (the exception, Ross at 12 m.s<sup>-1</sup> appeared to be levelling off at 90 seconds as the increase between 90 and 100 seconds was only from 85.9 to 88.0 l.min<sup>-1</sup>).

Virtually all of the variables measured at ten second intervals (with the single exception of  $P_{EO_2}$  in on case at 8 m.s<sup>-1</sup>) had reached a steady state after a minute at 8 m.s<sup>-1</sup> and by ninety seconds at 10 m.s<sup>-1</sup>. At 12 m.s<sup>-1</sup>, nearly all variables appeared to be at a steady state

by ninety seconds, the exceptions being  $\dot{V}\text{CO}_2$  in one horse and PEF in another, although in the latter case a plateau appeared to have been more or less reached by eighty seconds. During the canter at  $12 \text{ m.s}^{-1}$  on the  $3^\circ$  incline, the only alteration from steady state after eighty seconds was in  $P_{\text{E}}\text{CO}_2$  in one horse. Thus, with this exercise test, in the event of measurements made at the very end of two minutes being unsuitable for use, it would be valid to use an earlier sub-file obtained after at least ninety seconds of exercise, whatever the work load of the canter.

The findings of this study support those of Rose *et al.* (1990), who found that  $\dot{V}\text{O}_2$  and  $\dot{V}\text{CO}_2$  reached a steady state by ninety seconds after the beginning of strenuous exercise in a variety of protocols aimed at determining  $\dot{V}\text{O}_{2\text{max}}$ . Bayly *et al.* (1989) also found a steady state for  $\dot{V}\text{O}_2$  by two minutes during exercise at 74-111% of  $\dot{V}\text{O}_{2\text{max}}$ . Rose *et al.* (1990) pointed out that the response in the horse is faster than for man in whom  $\dot{V}\text{O}_2$  takes two minutes to reach steady state during supramaximal exercise (Åstrand and Saltin, 1961) and over six minutes for less intense exercise levels (Whipp, 1971).

In this study,  $P_{\text{a}}\text{O}_2$  was unaltered between one and two minutes of exercise, whereas  $P_{\text{a}}\text{CO}_2$  rose during the second minute (significantly in all canters but the slowest). Bayly *et al.* (1989) also found no change in  $P_{\text{a}}\text{O}_2$  during four minutes of exercise at 74-111% of  $\dot{V}\text{O}_{2\text{max}}$ , but a variable response by  $P_{\text{a}}\text{CO}_2$  which during the highest exercise level reached a peak after one to two minutes of exercise and thereafter fell to below resting values. Given that  $P_{\text{a}}\text{O}_2$  in both studies was unaltered with time whilst during strenuous exercise the greatest hypercapnic response found by Bayly *et al.* was at one to two minutes, the two minute sampling point would appear to be satisfactory for use in exercise testing.

Figure 6.5 Changes in respiratory frequency ( $\text{min}^{-1}$ ) with time for Thoroughbred horses during two minute canters on a treadmill

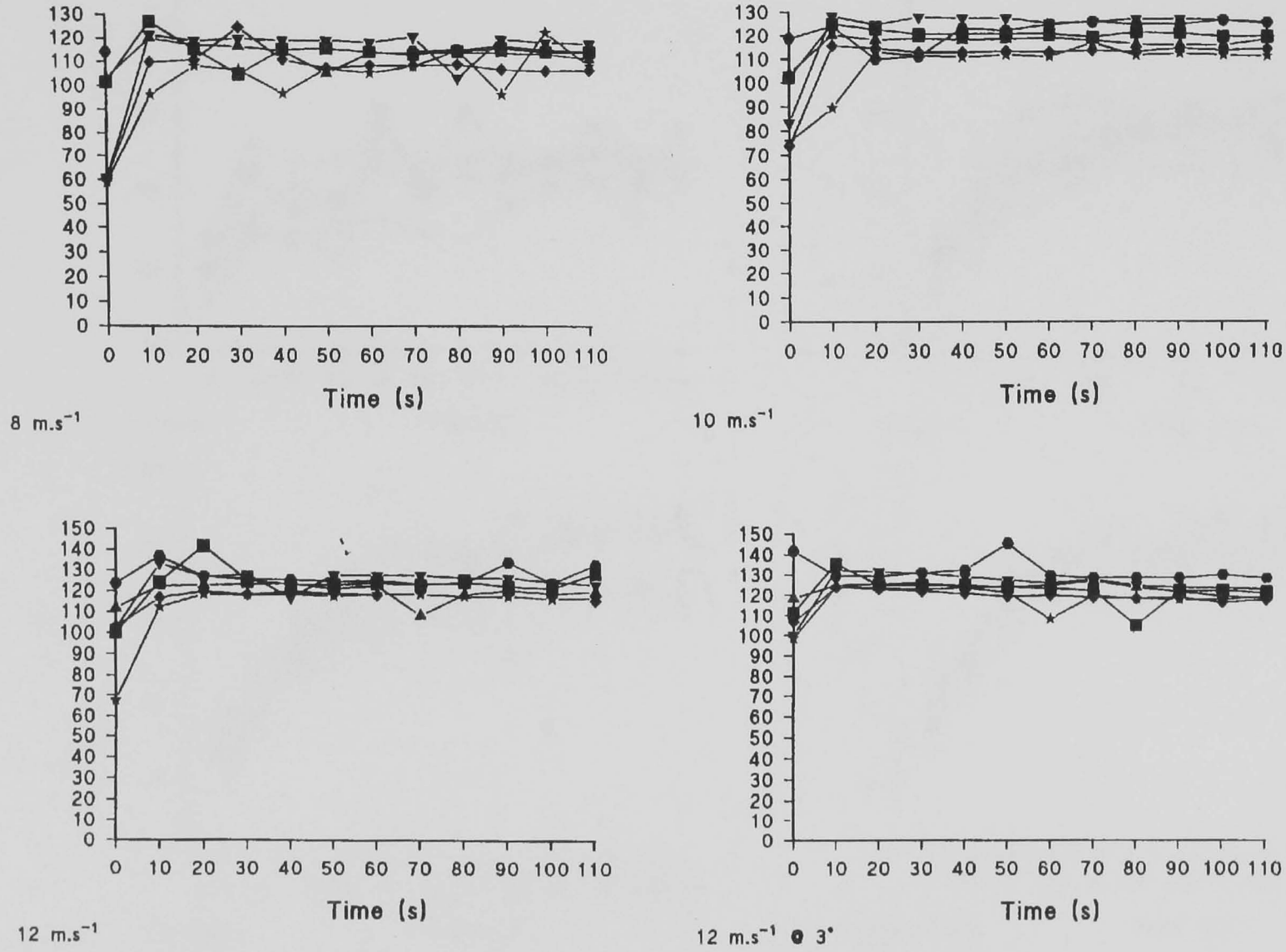
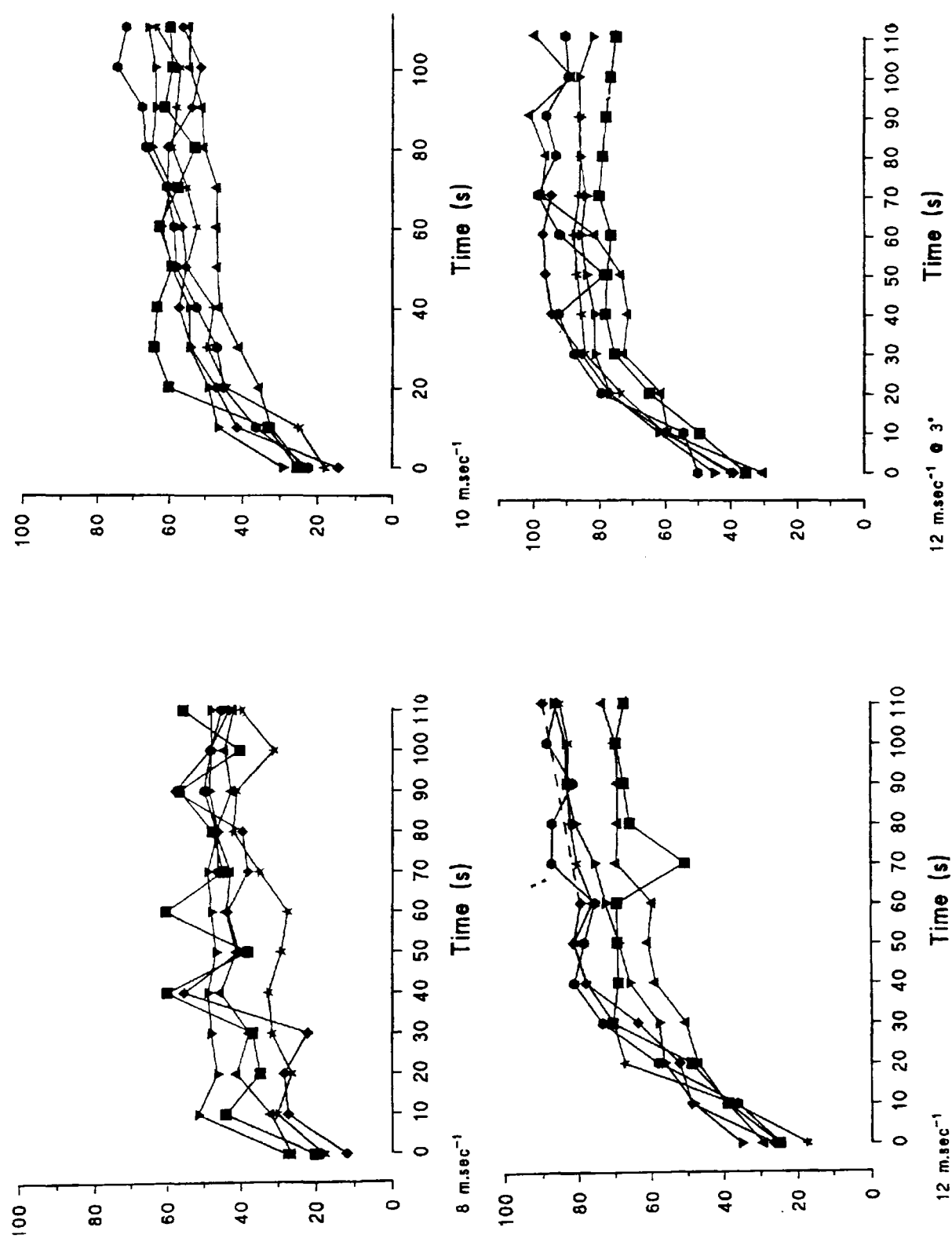


Figure 6.6 Changes in peak expiratory flow rate (l.s<sup>-1</sup>) with time for Thoroughbred horses during two minute canters on a treadmill



## **CHAPTER SEVEN - RESPIRATORY RESPONSES TO EXERCISE IN THE NORMAL THOROUGHBRED HORSE**

The kinetics of the respiratory response during fast exercise (i.e. during canter) in the Thoroughbred were described in the previous chapter. This one will consider the respiratory response to exercise at varying gaits and workloads.

### **Summary of Methods**

As in the previous Chapter, data are presented from the exercise tests performed on six Thoroughbred horses at the end of a fifteen week training programme. As a brief reminder, the test protocol consisted of ten minutes at walk ( $1.6 \text{ m.s}^{-1}$ ), three minutes at trot ( $3.2 \text{ m.s}^{-1}$ ) and five minutes at walk ( $1.6 \text{ m.s}^{-1}$ ), followed by four two minute canters (8, 10 and  $12 \text{ m.s}^{-1}$  on a level surface and  $12 \text{ m.s}^{-1}$  on a  $3^\circ$  incline) separated by ten minute walks ( $1.6 \text{ m.s}^{-1}$ ). For further details of the methods see Chapter Three.

As discussed in the previous chapter, data for canter exercise will be taken from the end of the two minute periods at this gait and blood gas determinations from the end of the second minute.

An analysis of variance for repeated measures was used to compare results from differing parts of the test, with differences being regarded as significant at the 0.05 level of probability.



**Results**

Data collected from six fully-trained, normal Thoroughbred horses during the standardised exercise test are presented in Figures 7.1-7.18.

*Resting values*

As the ultrasound flow transducer system is relatively insensitive to the flow rates which occur in resting horses when the gain is adjusted so that the high flow rates of fast exercise can be measured, data from resting horses could not be analysed satisfactorily in most cases. Accordingly, only blood gas data are given for resting horses.

Values for blood gases and pH at rest, corrected for body temperature, were as follows (mean  $\pm$  SEM):

	<i>Arterial</i>	<i>Mixed venous</i>
Po <sub>2</sub> (mm Hg)	94.1 $\pm$ 1.7	41.7 $\pm$ 1.8
S <sub>a</sub> O <sub>2</sub> (%)	97.5 $\pm$ 0.1	82.3 $\pm$ 1.4
Pco <sub>2</sub> (mm Hg)	46.9 $\pm$ 0.8	51.9 $\pm$ 0.9
pH	7.448 $\pm$ .005	7.430 $\pm$ .005

In an earlier reported study from this laboratory using the ultrasound flow transducer system for measurement of respiratory flows in Thoroughbred horses of comparable size (Butler *et al.*, 1993a) measurements were made of respiratory variables in resting horses following digitisation of recorded traces. Table 7.1 lists resting values for respiratory variables measured in that study, for comparison with exercise values of these parameters in this study.

**Table 7.1: Values for respiratory variables (mean  $\pm$  SEM) in Thoroughbred horses at rest (from Butler *et al.*, 1993a)**

$f_b$	13.5 ( $\pm$ 1.5) min <sup>-1</sup>
$V_t$	5.1 ( $\pm$ 0.5) l
$\dot{V}_e$	67.0 ( $\pm$ 8.1) l.min <sup>-1</sup>
$\dot{V}_A$	37.5 ( $\pm$ 5.3) l.min <sup>-1</sup>
$V_{dphys}/V_t$	0.41 ( $\pm$ 0.05)
PIF	3.4 ( $\pm$ 0.4) l.s <sup>-1</sup>
PEF	5.7 ( $\pm$ 0.5) l.s <sup>-1</sup>
$P_{ET}O_2$	103.5 ( $\pm$ 1.5) mm Hg
$P_{ET}CO_2$	49.5 ( $\pm$ 1.5) mm Hg
$C_aO_2$	20.8 ( $\pm$ 0.6) ml.dl <sup>-1</sup>
$C_{\bar{v}}O_2$	16.5 ( $\pm$ 0.8) ml.dl <sup>-1</sup>
[Hb]	15.1 ( $\pm$ 0.4) g.dl <sup>-1</sup>
$\dot{V}O_2$	4.7 ( $\pm$ 0.6) ml.min. <sup>-1</sup> kg <sup>-1</sup>
$\dot{Q}$	115.0 ( $\pm$ 20.7) ml.min. <sup>-1</sup> kg <sup>-1</sup>
$t_v$	37.2 ( $\pm$ 0.3) °C

*Exercise*

During exercise tests, respiratory frequency ( $f_b$ ) was much greater at all gaits than at rest (Figure 7.1).  $f_b$  was similar during trot to that reached during the initial walk, but increased significantly during the canters.  $f_b$  increased only slightly as canter speed increased, having mean values of 112.7 ( $\pm$ 1.9), 117.5 ( $\pm$ 2.8), 121.8 ( $\pm$ 2.0) and 121.7 ( $\pm$ 1.7) breaths per

minute at 8, 10, 12 m.s<sup>-1</sup> and 12 m.s<sup>-1</sup> on a 3° incline respectively<sup>1</sup>.  $f_b$  showed no significant differences between any of the canter speeds. Stride rate ( $f_s$ ) during the canters (determined by manual counting) was virtually identical to  $f_b$ .

During the later walks (W4 to W6)  $f_b$  was significantly higher than during the earlier walks and trot, reflecting the hyperventilation which was a progressively increasing feature of these later recovery periods (see below). Indeed, W4 was not significantly different from C8 and C10 nor W5 and W6 from all canters in this respect.

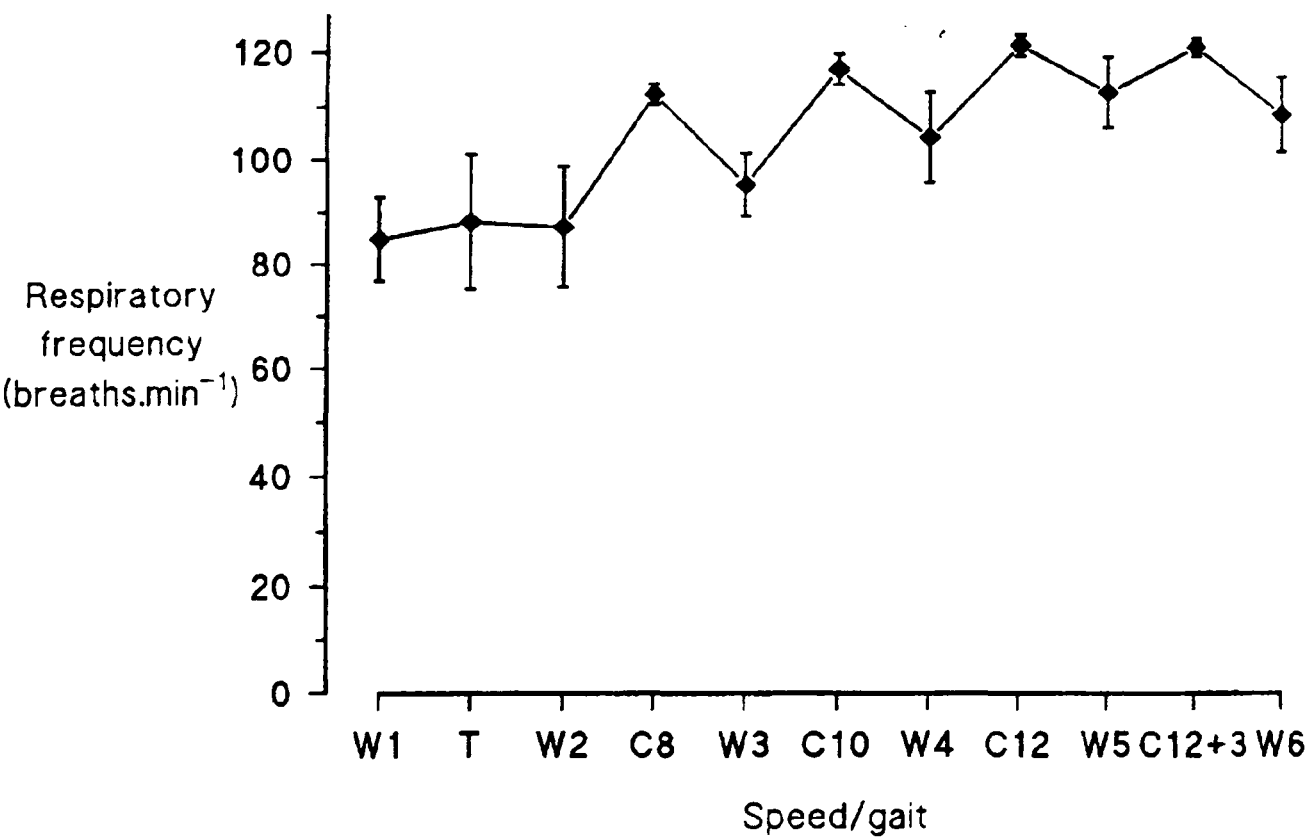
Expired tidal volume ( $V_t$ ) during the initial walk (mean 5.4 ± 0.35l) was similar to reported resting values but increased through trot and the increasing canter workloads to a maximum mean value of 16.6l (± 0.54) in C12+3 (Figure 7.2). In the final two recovery walks  $V_t$  tended to increase and was significantly higher in the final walk than in the earlier ones.

Minute ventilation ( $\dot{V}_e$ ), the product of  $f_b$  and  $V_t$ , increased substantially from walk to the highest workload (Figure 7.3), reaching a maximum mean value of 2,034.7l (± 70.1l), a 30.4 fold increase over the resting values previously reported in this laboratory (Butler *et al.*, 1993a - see Table 7.1).  $\dot{V}_e$  in the final two recovery walks was significantly greater than in the other walks and the trot.

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<sup>1</sup>Henceforth these canters are designated C8 (8 m.s<sup>-1</sup>), C10 (10 m.s<sup>-1</sup>), C12 (12 m.s<sup>-1</sup>) and C12+3 (12 m.s<sup>-1</sup> on a 3° incline). The periods of walk are designated W1 to W6.

**Figure 7.1: Respiratory frequency during exercise in Thoroughbred horses**

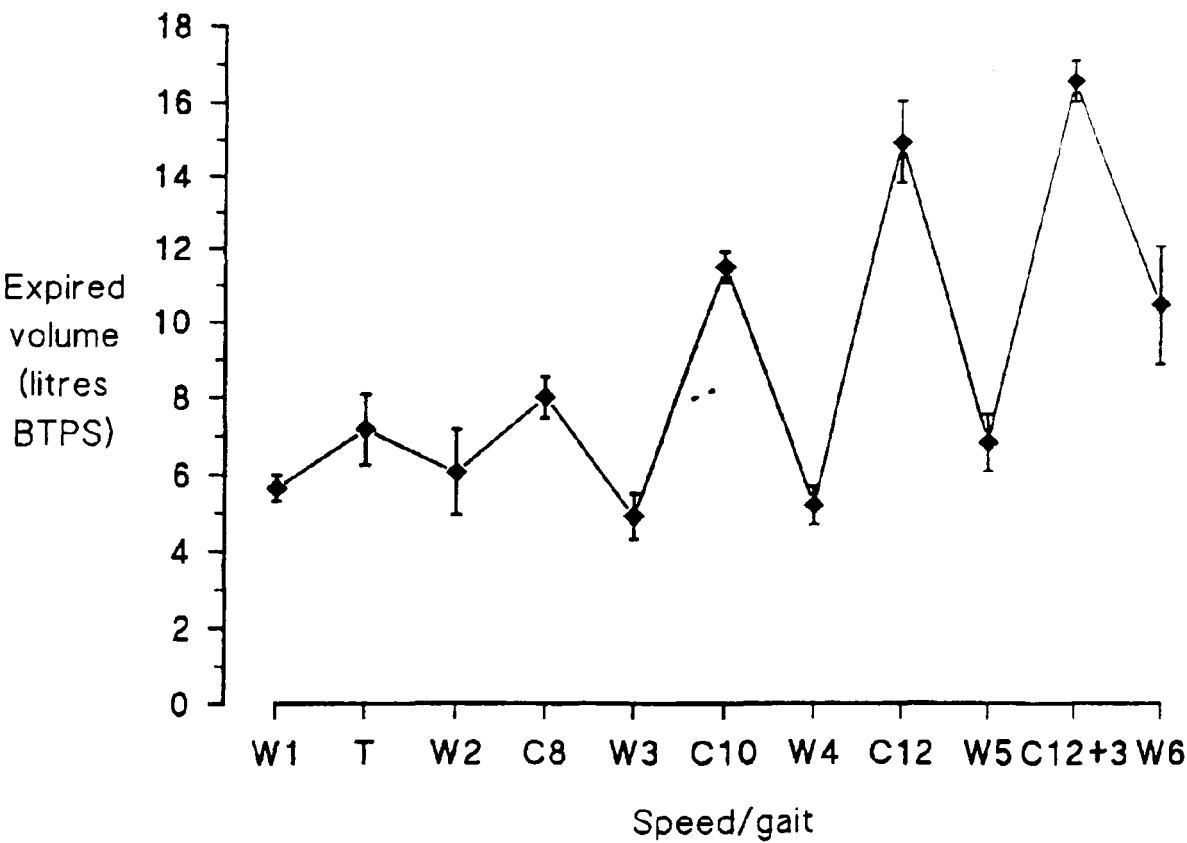


*In Figures 7.1-7.18 the following abbreviations apply:*

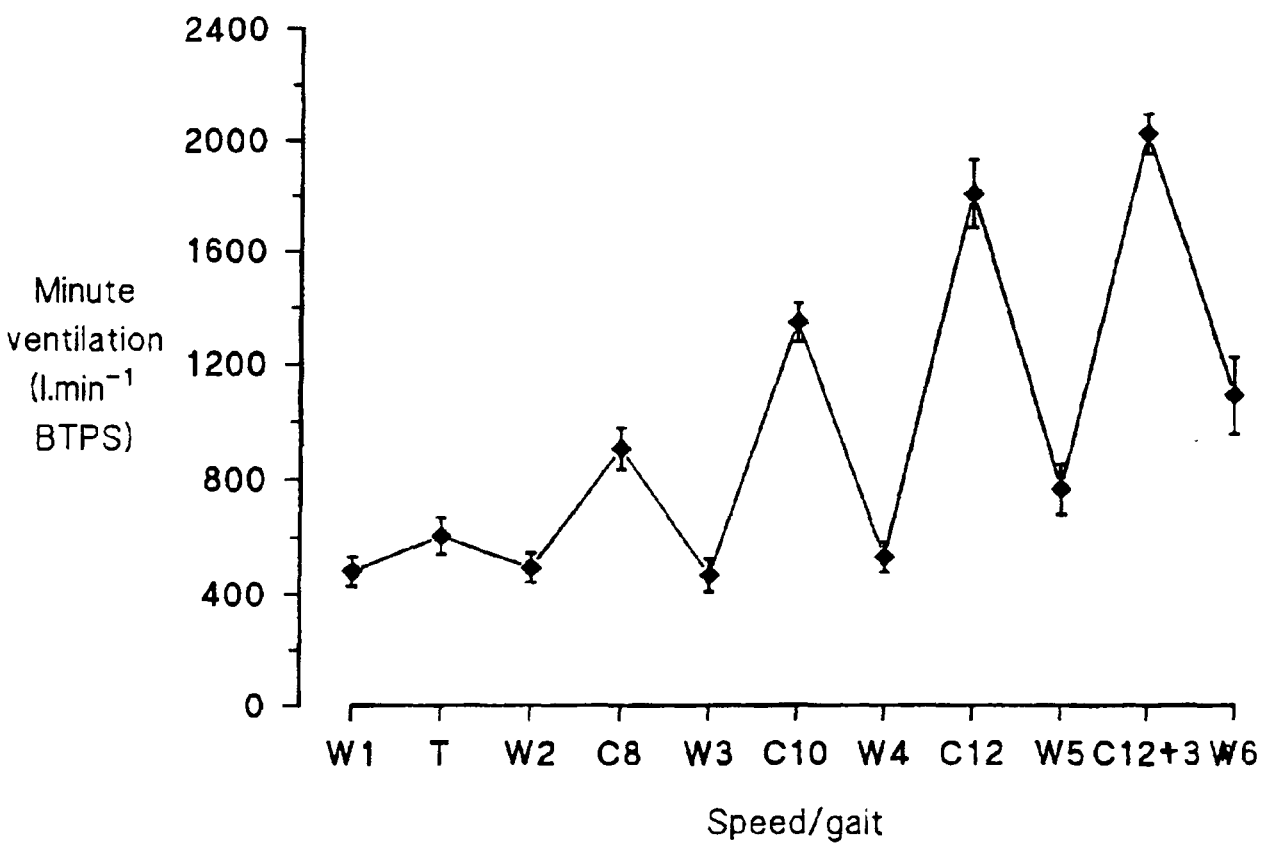
<i>W1 - W6</i>	<i>Walk periods</i>
<i>T</i>	<i>Trot</i>
<i>C8 - C12+3</i>	<i>Canter</i>

*Values are shown as mean ± SEM*

**Figures 7.2: Expired tidal volume during exercise in Thoroughbred horses**



**Figures 7.3: Minute ventilation during exercise in Thoroughbred horses**



The mean times taken for inspiration ( $t_i$ ) and expiration ( $t_e$ ) (Figure 7.4) during the initial walk were similar at 0.448s ( $\pm 0.076$ ) and 0.430s ( $\pm 0.062$ ) respectively. As  $f_b$  increased, these inevitably reduced, to minimum values of 0.236s ( $\pm 0.005$ ) and 0.257s ( $\pm 0.003$ ), at which point  $t_e$  was significantly greater than  $t_i$ . The ratio  $t_e/(t_i+t_e)$  showed no systematic variation (Figure 7.4a).

The ratio of these variables ( $t_i/t_e$ ) fluctuated about unity with the value at C12+3 being the lowest and significantly lower than in W1 and W2 (Figure 7.4).

Mean inspiratory flow rate (MIF) and peak inspiratory and expiratory flow rates (PIF and PEF) all increased significantly with each increment of speed (Figure 7.5). PEF was higher than PIF at all canter speeds and this difference was significant at speeds of 10 m.s<sup>-1</sup> and greater.

From C8 to C12, the increase was linear for all three flow rate variables, and MIF and PIF also increased significantly from C12 to C12+3. In contrast, PEF did not change significantly between C12 and C12+3. At the highest work load, PEF could not be calculated for one horse (Monty - who had the highest value for PEF at C12) due to inversion of the flow signal, suggesting peak flows out of the range of the transducers. Although missing the value for this horse would seem likely to have artificially reduced the mean value for PEF at C12+3 somewhat, three of the other five horses showed little change in PEF between C12 and C12+3 (changes of 1.6, 1.1 and -0.8 l.sec<sup>-1</sup>) suggesting that in some of the animals PEF was approaching a maximum at C12 and C12+3.

Figure 7.4:  $t_i$ ,  $t_e$  and  $t_i/t_e$  during exercise in Thoroughbred horses

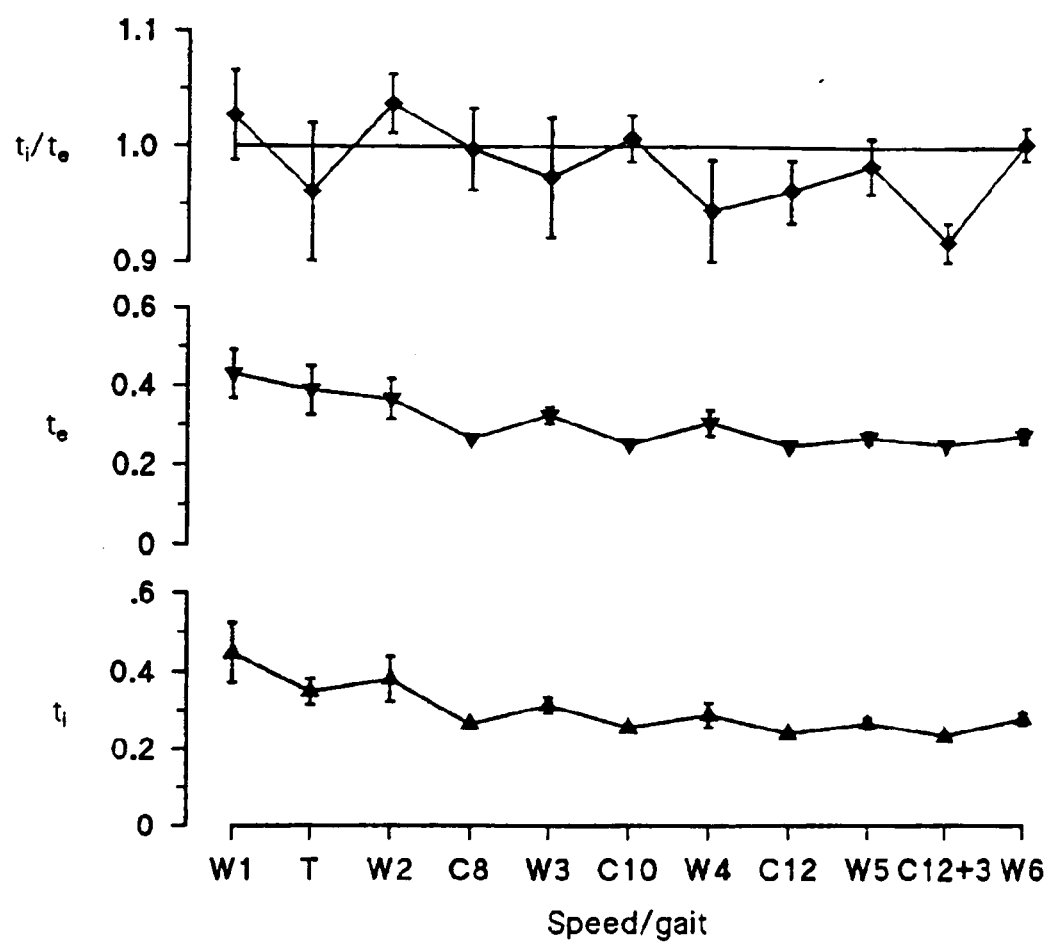
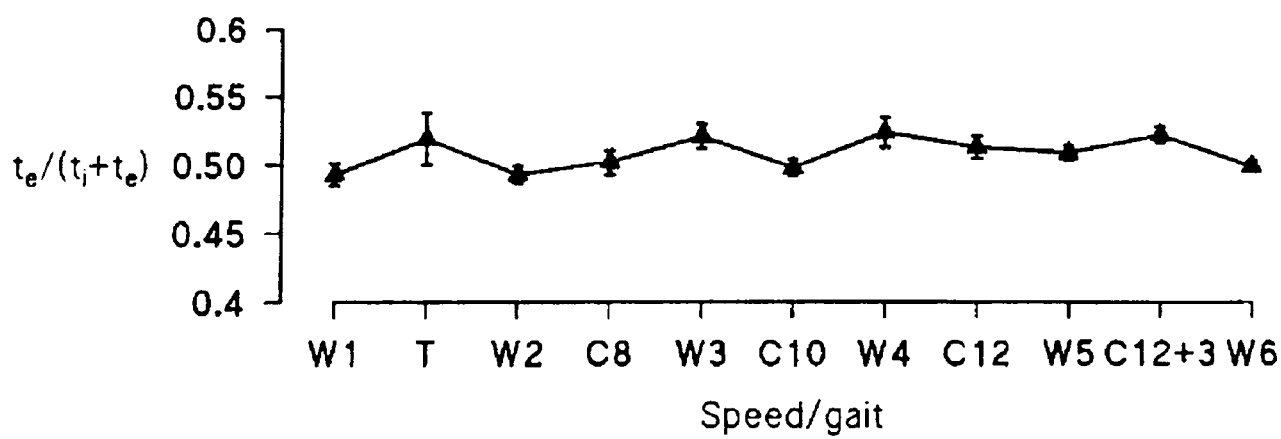
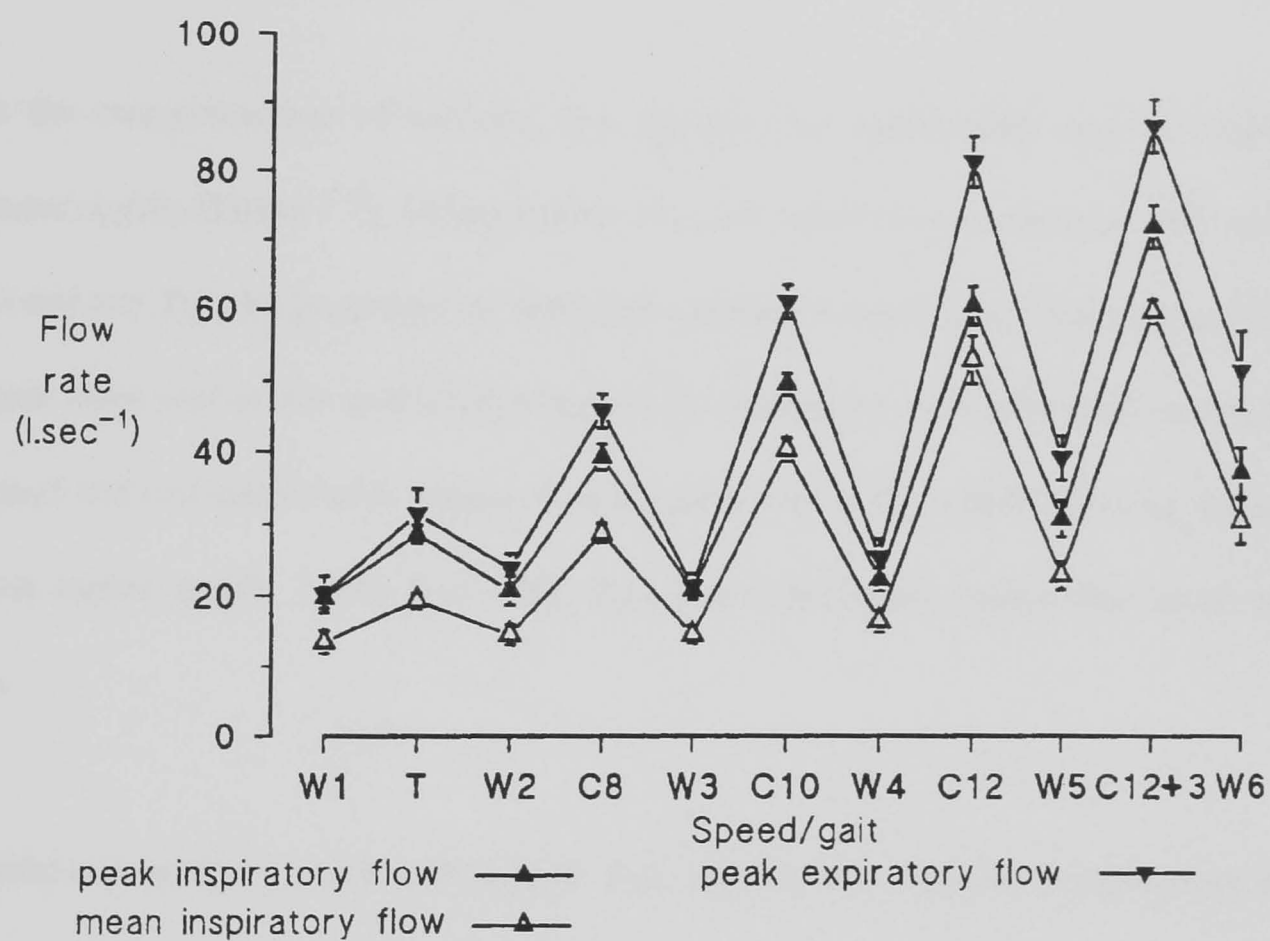


Fig 7.4a:  $t_e/(t_i+t_e)$  during exercise in Thoroughbred horses





Figures 7.5: Respiratory flow rates during exercise in Thoroughbred horses

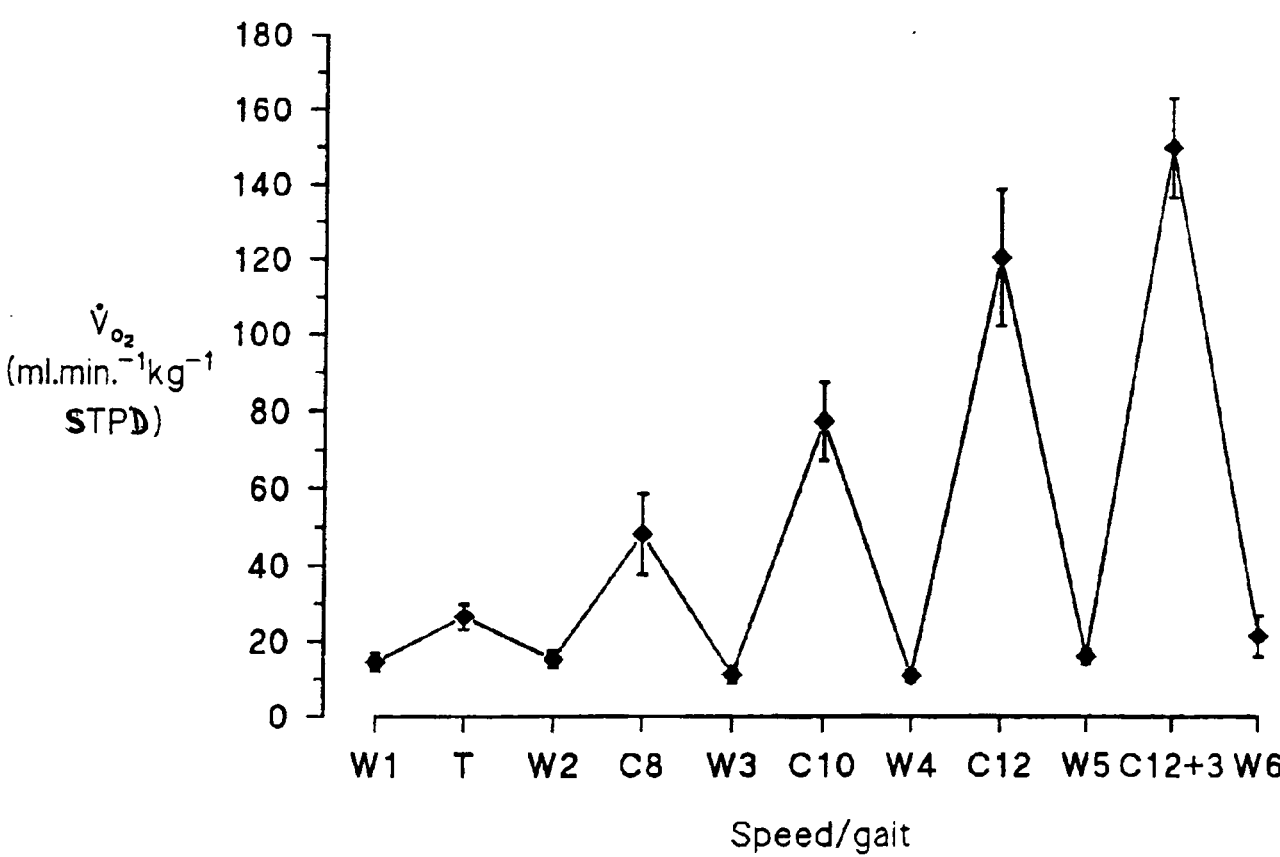


Oxygen consumption ( $\dot{V}O_2$ ) increased significantly with workload (Figure 7.6), reaching a mean value of  $150.8 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  ( $\pm 13.31$ ) at C12+3, a 32 fold increase over previously reported resting values from this laboratory (Butler *et al.*, 1993a - Table 7.1). The increase in  $\dot{V}O_2$  was linear with increasing workload over the range in which increase in workload was, itself linear (i.e. between C8 and C12). Changes in  $\dot{V}CO_2$  were similar to those in  $\dot{V}O_2$ ,  $\dot{V}CO_2$  being slightly higher at all exercise levels.

With the commencement of exercise,  $P_{aO_2}$  did not alter significantly until W2, when it increased slightly (Figure 7.7). During canter,  $P_{aO_2}$  fell significantly compared with rest, all walks and trot.  $P_{aO_2}$  fell progressively with each increase in work load, until during C12+3 its mean value was as low as  $60.2 \text{ mm Hg}$  ( $\pm 3.0$ ). During the walk after each canter,  $P_{aO_2}$  increased and was significantly greater than at rest or trot in the walks following the three highest canter speeds. In the final walk,  $P_{aO_2}$  was significantly greater than at all other times.

End-tidal and mixed expired  $PO_2$  ( $P_{ETO_2}$  and  $P_{EO_2}$  respectively) declined progressively with increased work level above trot and walk respectively (Figure 7.8).  $P_{ETO_2}$  returned to W1 levels during the recovery walks and was elevated during W6, whilst  $P_{EO_2}$  was slightly elevated during all canter recoveries. The end-tidal-arterial  $PO_2$  difference ( $P_{(ET-a)O_2}$ ) was  $10.7 \text{ mm Hg}$  ( $\pm 1.7$ ) during W1 and increased as workload increased as follows:

**Figures 7.6: Changes in oxygen consumption during exercise in Thoroughbred horses**



Figures 7.7: Changes in  $P_{O_2}$  during exercise in Thoroughbred horses

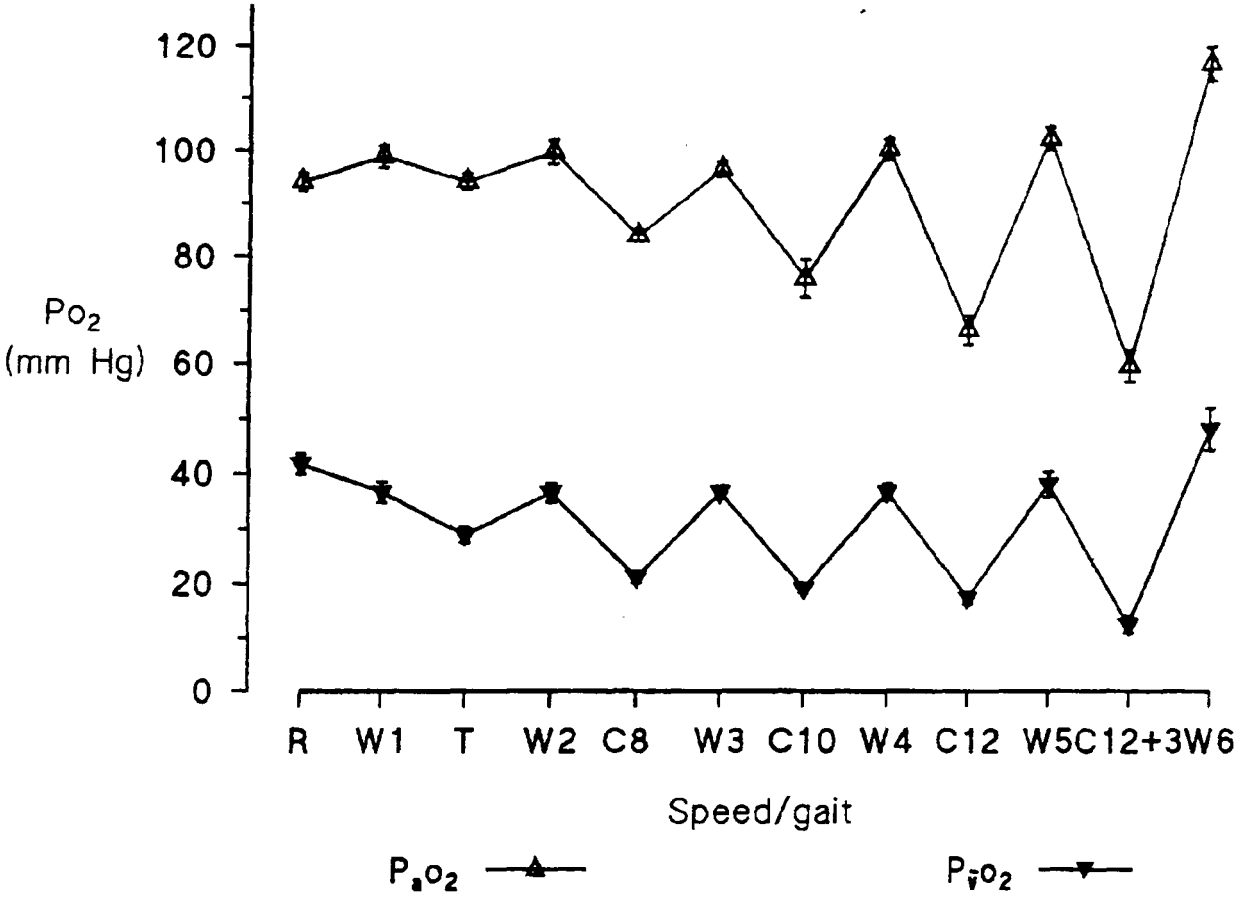
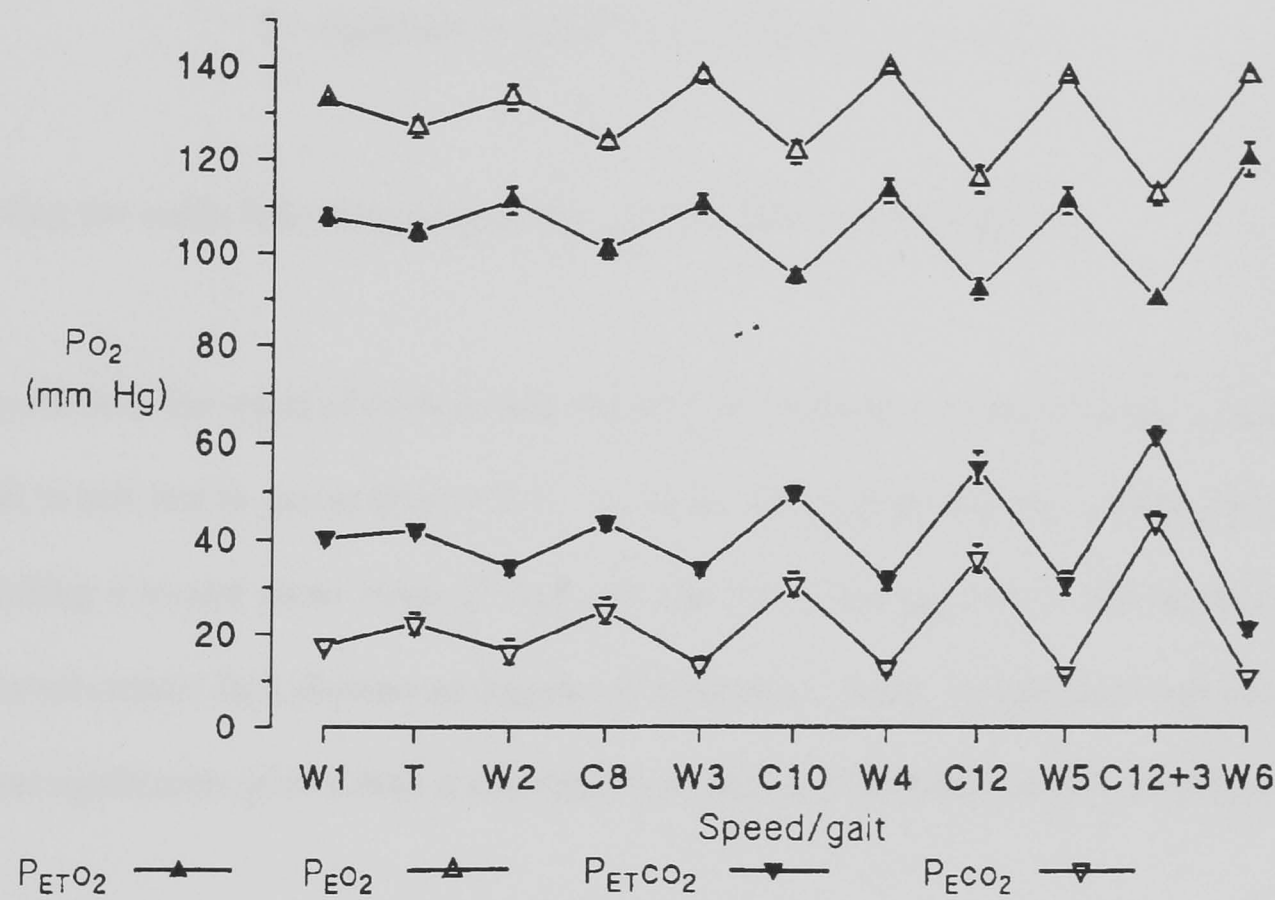


Figure 7.8: Expired gas tensions during exercise in Thoroughbred horses



Trot	13.2 mm Hg ( $\pm 1.6$ )
C8	21.6 mm Hg ( $\pm 1.4$ )*¶
C10	24.9 mm Hg ( $\pm 2.8$ )*
C12	32.2 mm Hg ( $\pm 1.9$ )*¶
C12+3	36.1 mm Hg ( $\pm 6.5$ )*

\* = significantly different from W1

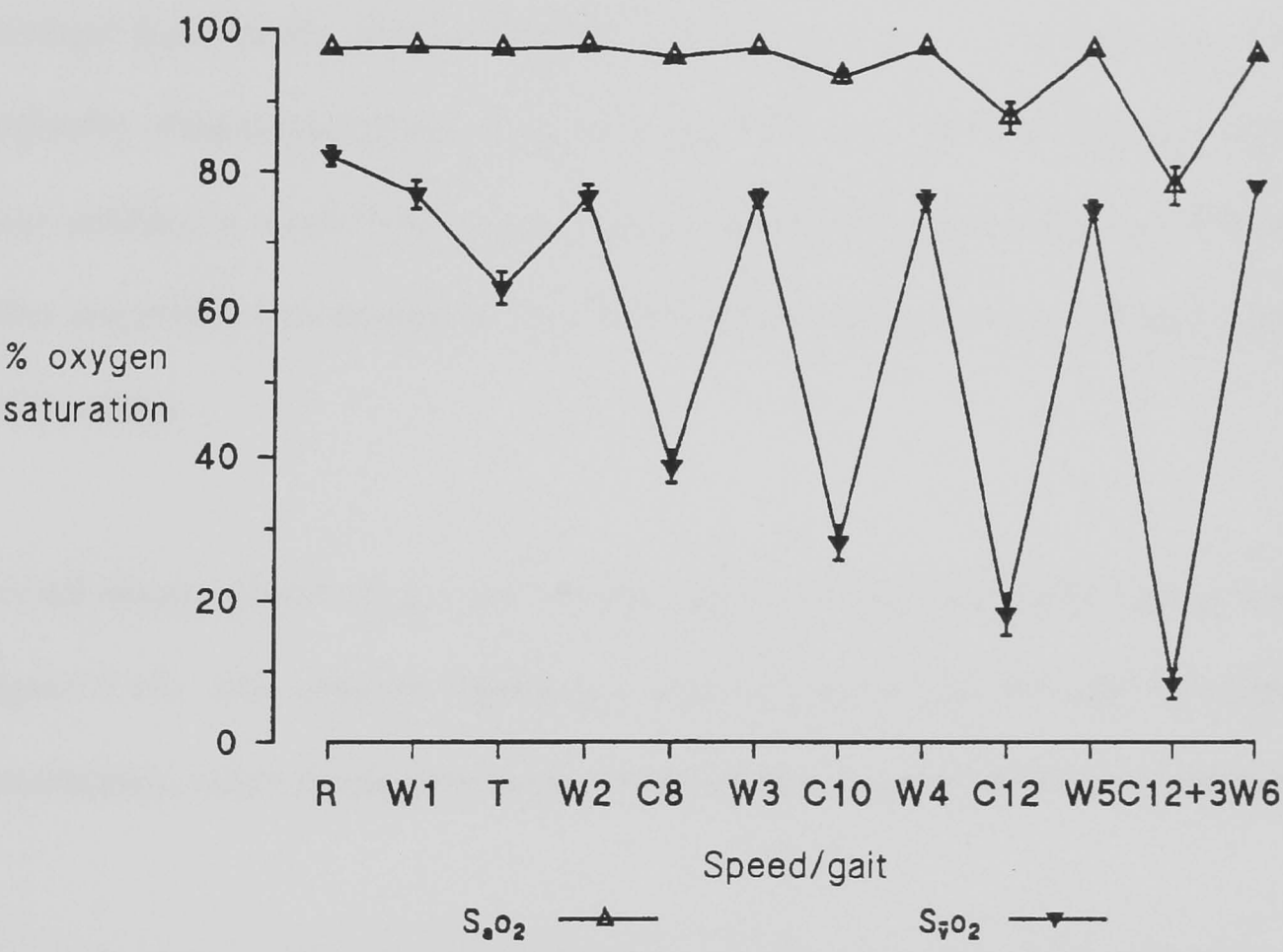
¶ = significantly different from previous work level

During the walks following canters,  $P_{(ET-a)}O_2$  was similar to during W1.

$P_{\bar{v}}O_2$  fell with the onset of exercise and reduced significantly with each change of gait, i.e. walk to trot, trot to canter (Figure 7.7). As canter speed increased,  $P_{\bar{v}}O_2$  continued to fall, reaching a lowest mean value of 12.6 mm Hg ( $\pm 1.5$ ) during C12+3. During the walks between canters,  $P_{\bar{v}}O_2$  showed no significant differences except for the final walk in which it was significantly greater than at any other time, with a mean value of 48.5 mm Hg ( $\pm 3.9$ ).

Due to the shape of the haemoglobin-oxygen dissociation curve (see Discussion), arterial haemoglobin oxygen saturation ( $S_aO_2$ ) showed little change during exercise until C10 (Figure 7.9). At the higher speeds progressively greater reductions occurred, the lowest value being 78.4% ( $\pm 2.7$ ). Mixed venous blood haemoglobin oxygen saturation ( $S_{\bar{v}}O_2$ ) showed a reduction at each increment of work intensity and was lowest during C12+3, when it was as low as 8.3% ( $\pm 2.1$ ).

Figure 7.9: Changes in blood oxygen saturation during exercise in Thoroughbred horses



With the onset of exercise, packed cell volume and hence also blood haemoglobin concentration rose, continuing to increase with workload until, at C12+3, maximum mean values of 68.1% and 23.3 g/dl were reached, representing an increase in oxygen carrying capacity of approximately 41% (Figure 7.10).

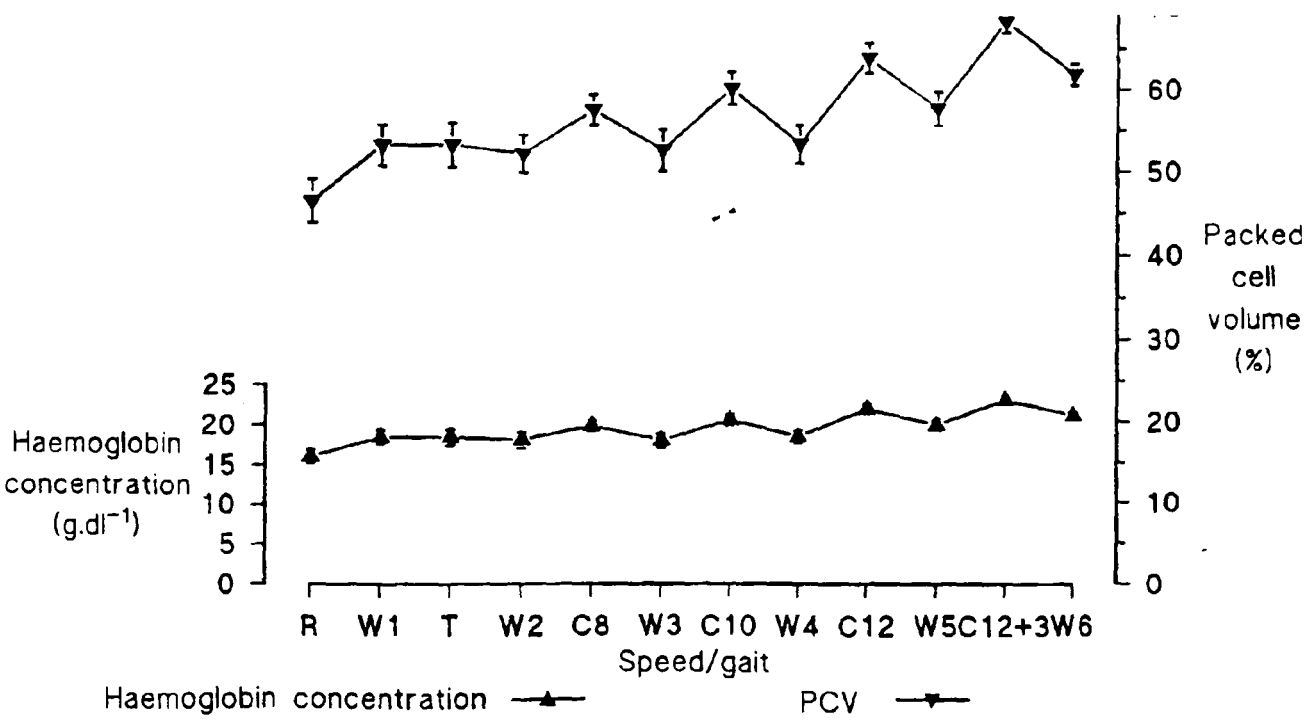
Measured values for blood oxygen content were found to be unreliable and hence values calculated from the  $P_{O_2}$  and haemoglobin concentration were used. The reason for the unreliability of the measured blood oxygen content data using Tucker's method (which has given satisfactory results in the past) could not be determined as unfortunately since the author was involved in running the exercise tests it was necessary for a volunteer to carry out the assays.

Arterial oxygen content ( $C_{aO_2}$ ) was increased above resting values at all exercise speeds (Figure 7.11). This occurred largely as a result of the increase in blood haemoglobin concentration, which during the canter countered the effects of the arterial hypoxaemia.

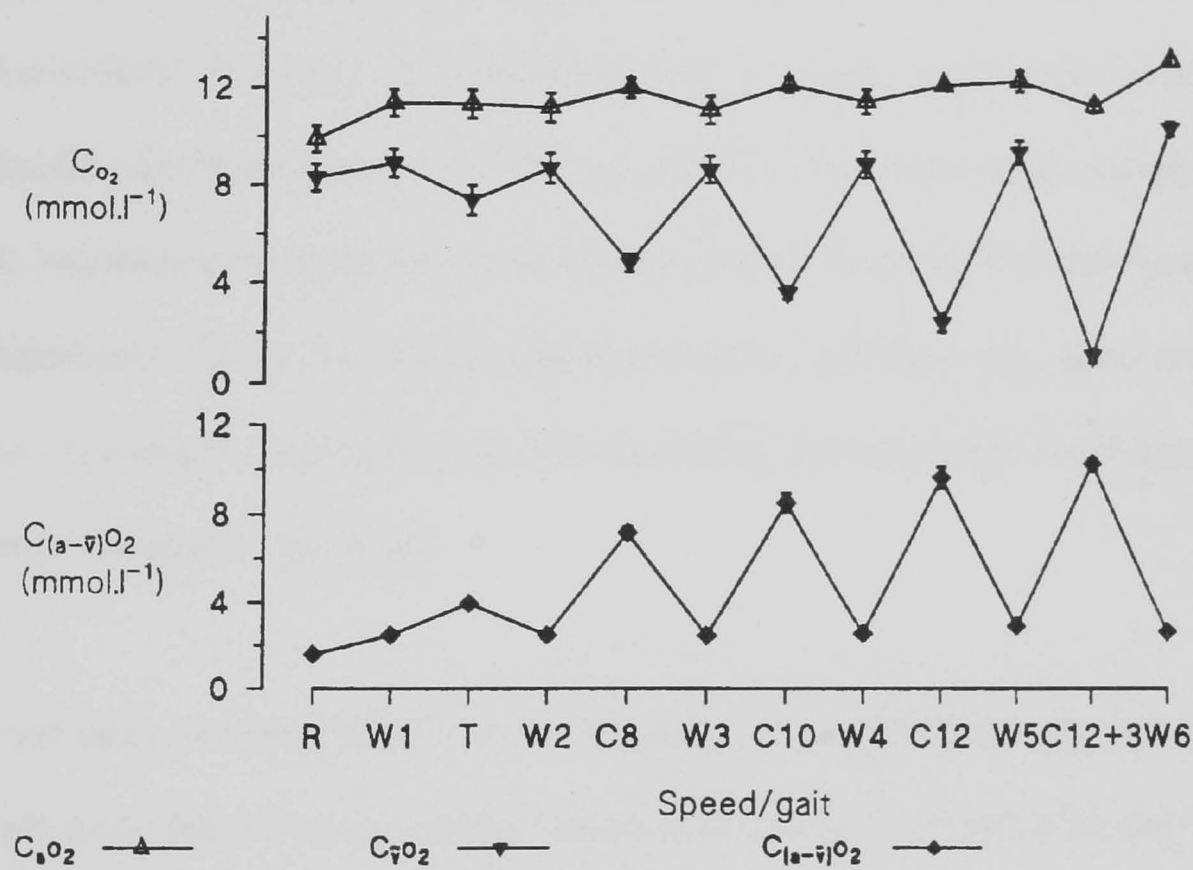
As a result of this mechanism,  $C_{aO_2}$  remained constant between C8 and C12 despite a fall of 15.7 mm Hg in mean  $P_{aO_2}$  between these exercise levels. The lowest mean value for  $C_{aO_2}$  during canter was that seen at C12+3, however even that value, occurring with a mean  $P_{aO_2}$  of 60.2 mm Hg, was significantly greater than at rest.



**Figure 7.10: Changes in blood haemoglobin concentration and packed cell volume during exercise in Thoroughbred horses**



Figures 7.11: Changes in blood oxygen contents during exercise in Thoroughbred horses



Mixed venous blood oxygen content ( $C_{\bar{v}O_2}$ ) changed little between rest and walk, but showed a progressive reduction as workload increased (Figure 7.11). As a result of this, arterial-mixed venous blood oxygen content difference ( $C_{a-\bar{v}O_2}$ ) increased from 1.58 ( $\pm 0.11$ ) mmol.l<sup>-1</sup> at rest to 10.37 ( $\pm 0.24$ ) mmol.l<sup>-1</sup> at C12+3 (Figure 7.11).

$P_aCO_2$  was elevated above resting levels during C10, C12 and C12+3, arterial hypercapnia becoming more marked over these work levels (Figure 7.12). The maximum value for  $P_aCO_2$  was 63.4 mm Hg ( $\pm 3.4$ ) during C12+3. During W4-W6,  $P_aCO_2$  was significantly lower than at rest. End-tidal and mixed expired  $P_{CO_2}$  ( $P_{ET}CO_2$  and  $P_ECO_2$ ) increased progressively with workload, becoming significantly elevated compared with W1 at C8 and C10 for  $P_ECO_2$  and  $P_{ET}CO_2$  respectively (Figure 7.8). The arterial-end-tidal  $P_{CO_2}$  difference ( $P_{(a-ET)CO_2}$ ) was 4.6 mm Hg ( $\pm 1.2$ ) during W1 and reduced towards zero during trot and canter, the change was not however statistically significant.

Arterial pH ( $pH_a$ ) increased slightly at the beginning of exercise. Both  $pH_a$  and mixed venous pH ( $pH_{\bar{v}}$ ) fell during the canters becoming as low as 7.130 ( $\pm 0.052$ ) and 7.008 ( $\pm 0.060$ ) respectively (Figure 7.13). Between canters values returned to pre-canter levels after C8 and C10, but were slightly reduced still in W5 after C12 and markedly reduced during W6 following C12+3.

Figures 7.12: Changes in  $P_{CO_2}$  during exercise in Thoroughbred horses

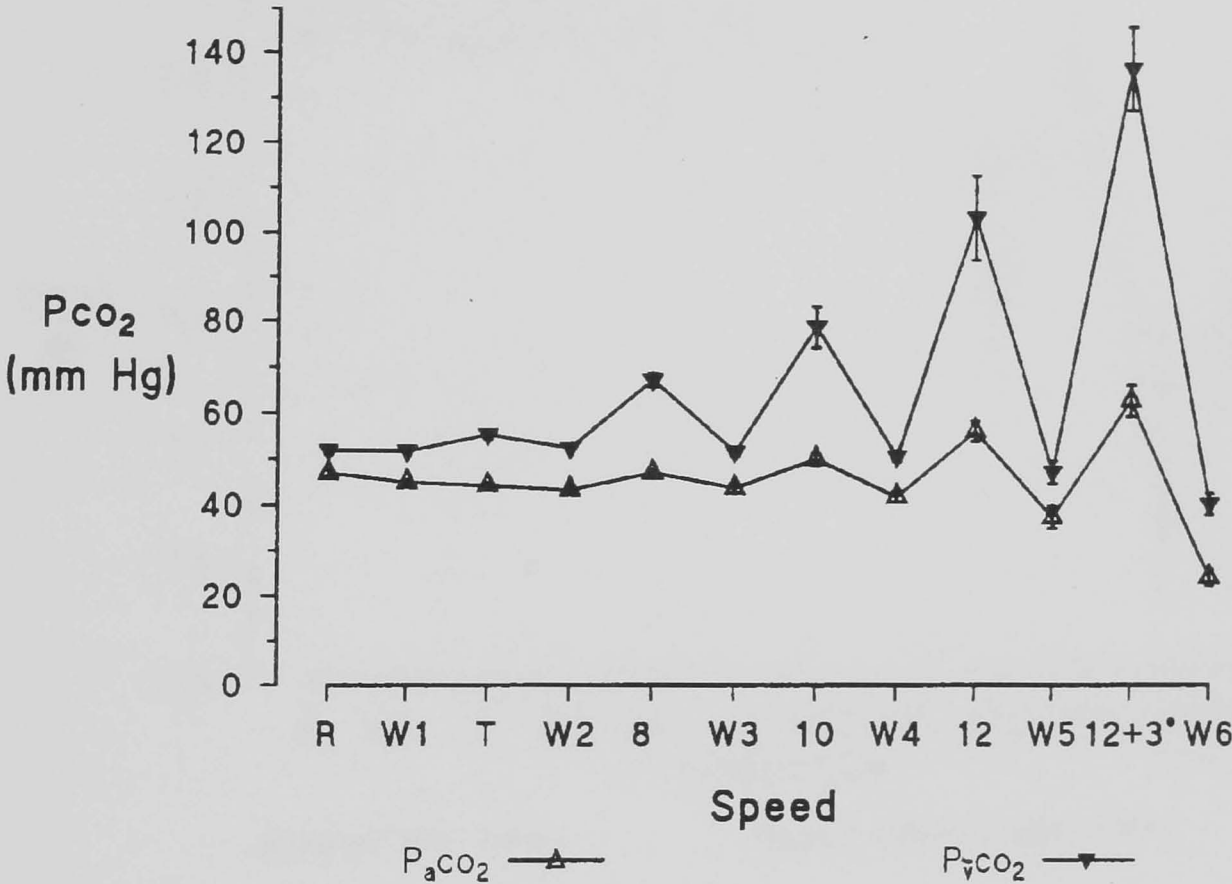
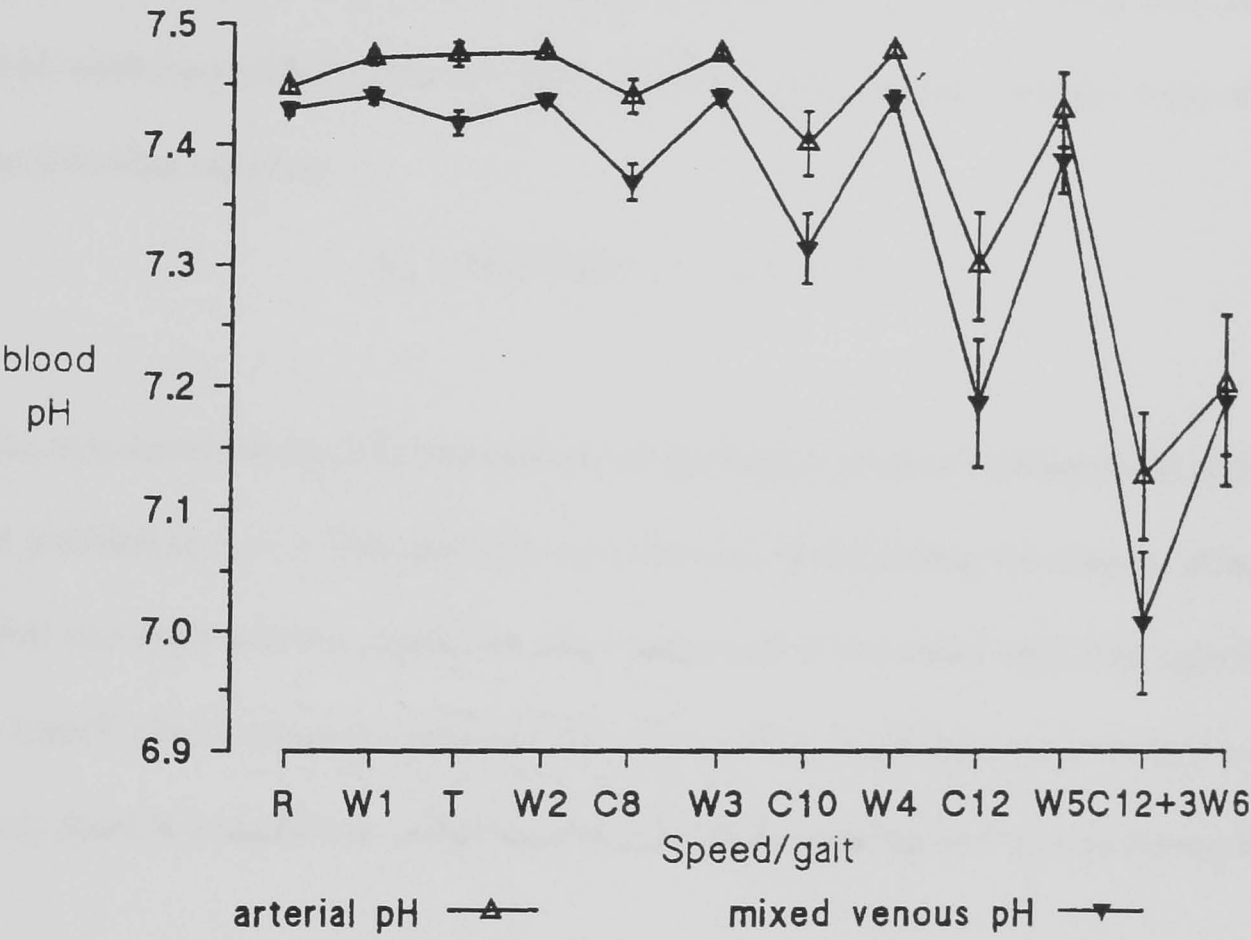


Figure 7.13: Changes in pH during exercise in Thoroughbred horses



Alveolar ventilation ( $\dot{V}_A$ ) increased with workload, the increase over walking levels becoming significant with the onset of canter and thereafter showing significant increases at each workload.

During the final walk,  $\dot{V}_A$  was higher than in all previous walks and trot, being slightly greater than during C8.  $\dot{V}_A$  increased linearly in relation to  $\dot{V}_{O_2}$  ( $r = 0.998$ ) over the full range of work loads (C8-C12) in this study (Figure 7.14). The two variables were related by the following equation:

$$\dot{V}_A = 20.0502(\dot{V}_{O_2}) + 112.3704$$

$\dot{V}_A/\dot{V}_{CO_2}$  was determined as a further estimate of the relative level of ventilation (in addition to that provided by  $P_aCO_2$ ). This ratio showed a tendency to fall during the canters, although the effect was slight and not significant when compared to the initial walk (but significant for C12 and C12+3 compared to most of the other walks). In the last two recovery walks,  $\dot{V}_A/\dot{V}_{CO_2}$  showed a significant and progressive increase reaching  $43.9 (\pm 4.6)$  during W6.

Physiological respiratory dead space volume ( $V_{dphys}$ ) was calculated using the Bohr equation. As a proportion of the total ventilation,  $V_{dphys}$  represented  $60.8\% (\pm 2.7\%)$  during the initial walk (Figure 7.15). During the higher work levels of trot and canter,  $V_d/V_t$  reduced progressively to a minimum of  $30\% (\pm 4.7\%)$  at C12+3. The ratio increased during the recovery walks compared to the canters that they followed and in all except W6 was significantly higher than during W1.

Cardiac output ( $\dot{Q}$ ), determined using the Fick principle, increased progressively during canter to a maximum of 641.6 ml.min.<sup>-1</sup>kg.<sup>-1</sup> ( $\pm 61$ ) during C12+3 (Figure 7.16). This represents a 5.6 fold increase over the resting value of 115.0 ml.min.<sup>-1</sup>kg.<sup>-1</sup> reported for resting horses in earlier studies (Butler *et al.*, 1993a - Table 7.1).  $\dot{Q}$  at the end of Walk 2 (end of warm up) was similar to the level measured during C8, but this may have been due to excitement prior to the first canter (horses not infrequently become more 'settled' following the first canter of an exercise test).

Mixed venous temperature ( $t_{\bar{v}}$ ) increased with each speed increment to reach a peak value of 40.4°C ( $\pm 0.5$ ) during C12+3 (Figure 7.17).

**Figure 7.14: Relationship between alveolar ventilation and oxygen consumption for Thoroughbred horses**

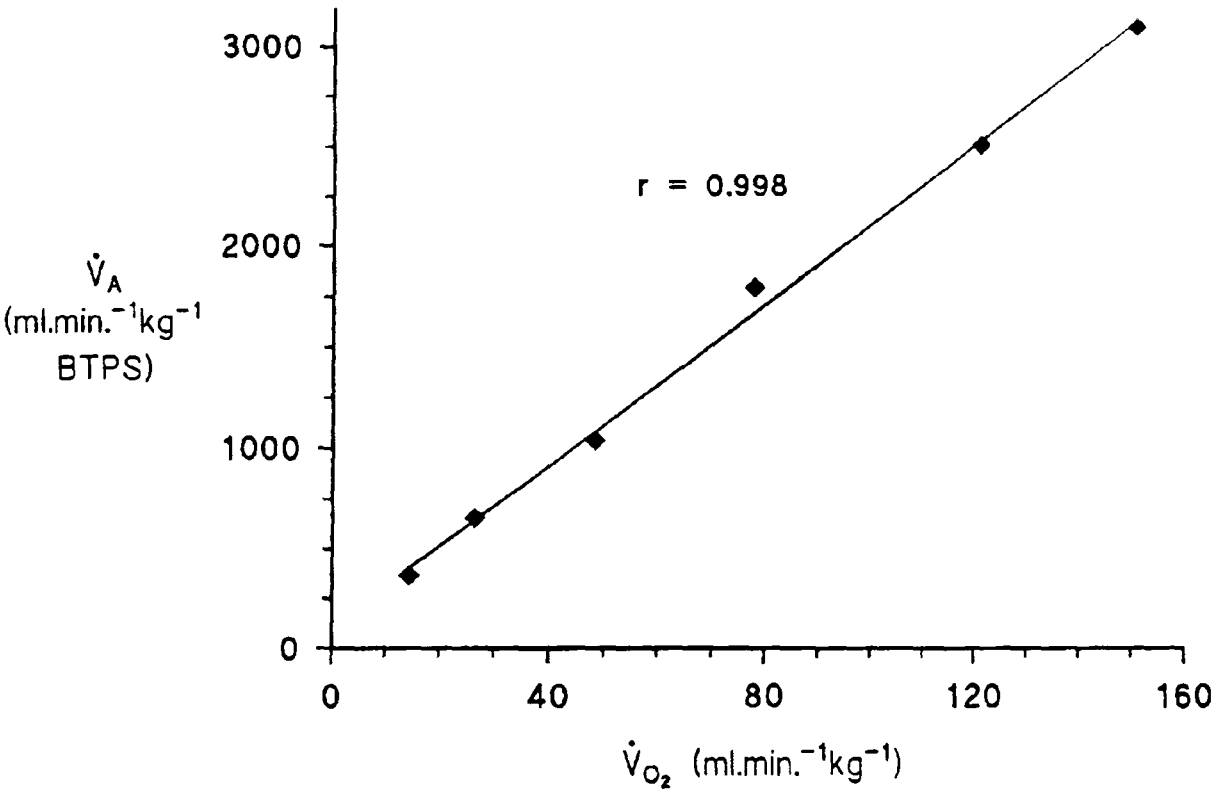
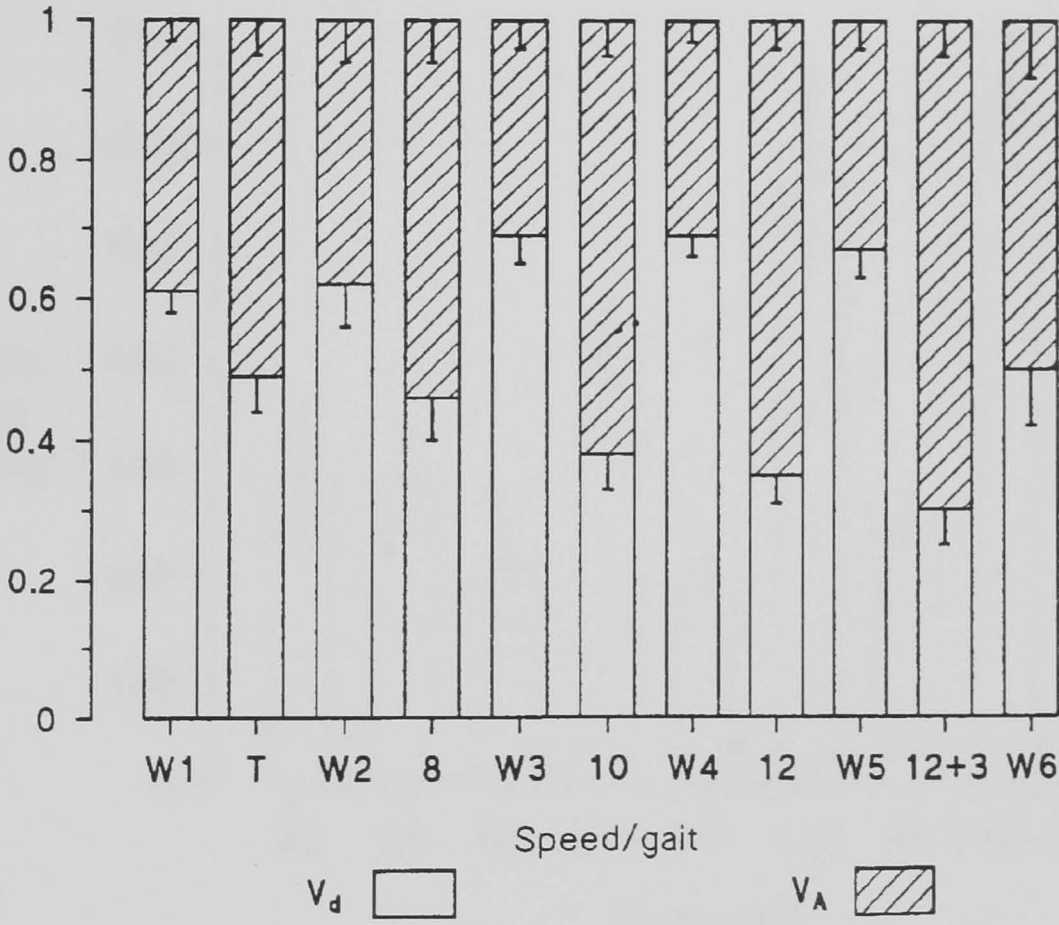
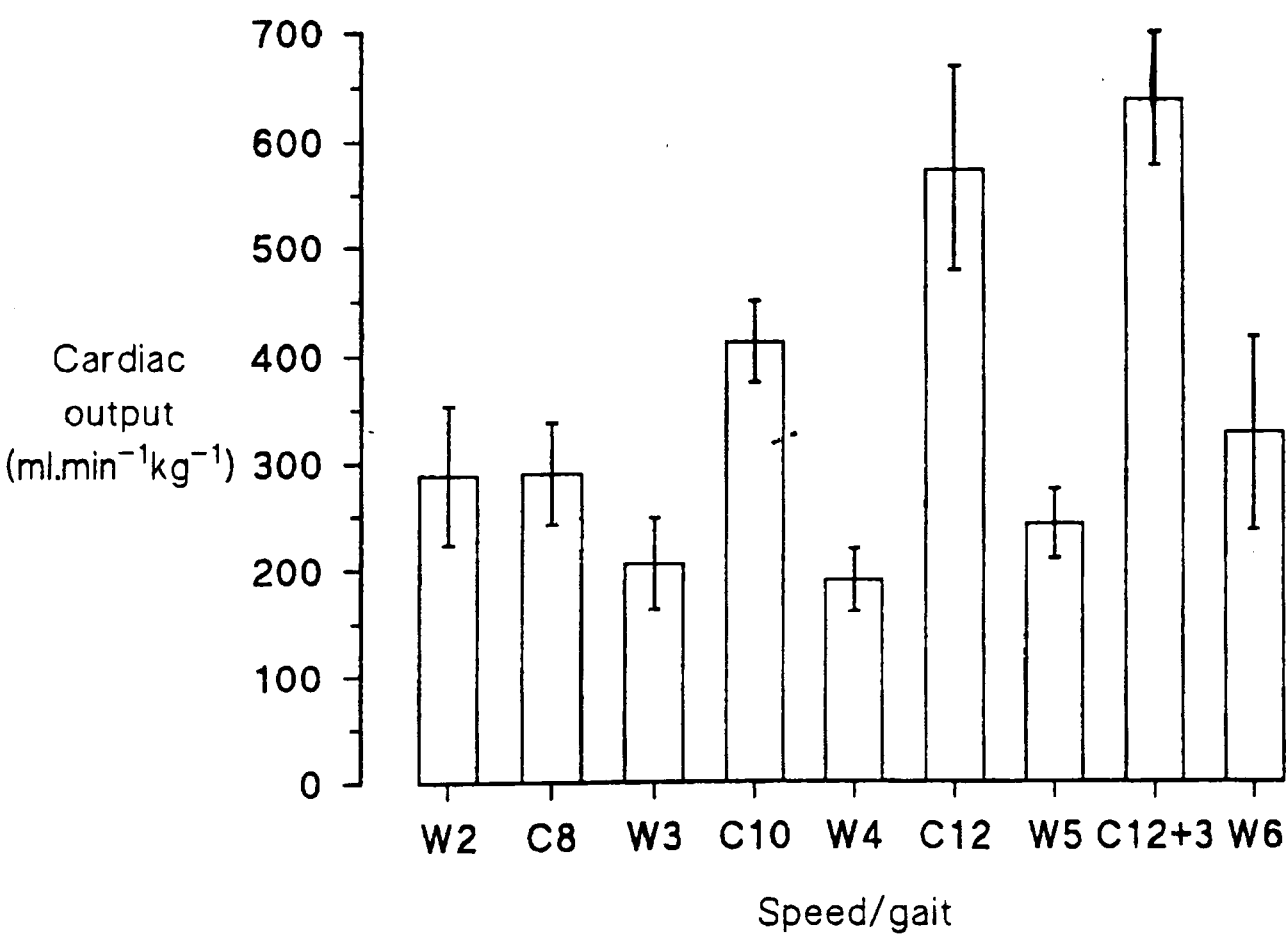




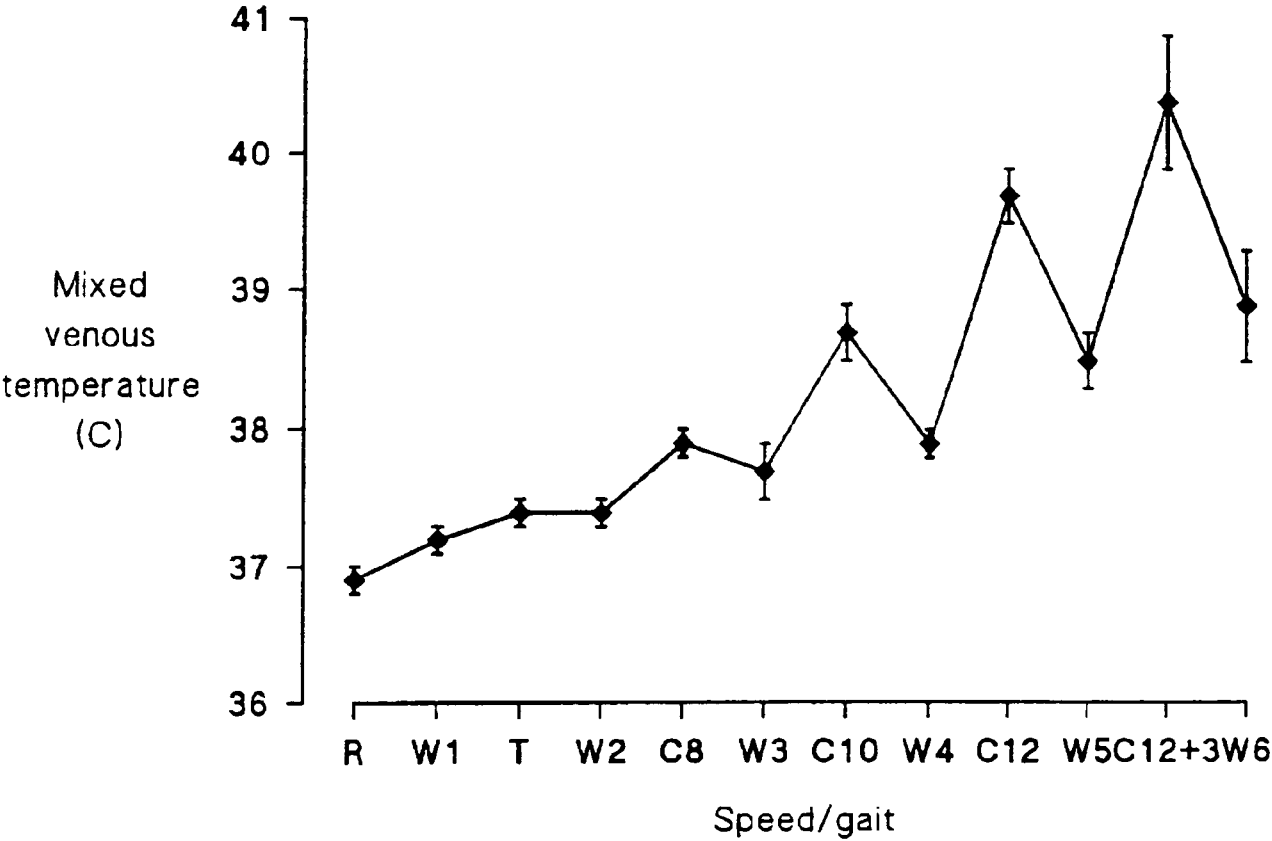
Figure 7.15: Proportions of  $V_d$  and  $V_A$  during exercise in Thoroughbred horses



**Figure 7.16: Cardiac output during exercise in Thoroughbred horses**



**Figure 7.17: Mixed venous temperature during exercise in Thoroughbred horses**



## Discussion

Respiratory responses to exercise by Thoroughbred horses undergoing treadmill exercise have been described several times previously (e.g. Hörnicke *et al.*, 1983; Bayly *et al.*, 1987a; Evans and Rose, 1988b; ; Rose and Evans, 1987; Butler *et al.*, 1993a). The responses shown by the horses in this test are similar to those described previously and in general of similar orders of magnitude. A few comments are however in order.

In view of the dramatic changes in blood gas tensions and pH associated with strenuous exercise in the Thoroughbred horse, reported here and in earlier studies (Bayly *et al.*, 1983; Thornton *et al.*, 1983; Bayly *et al.*, 1987a; Evans and Rose, 1988a and 1988b; Littlejohn and Snow, 1988; Anderson *et al.*, 1989; Bayly *et al.*, 1989; Wagner *et al.*, 1989; Art *et al.*, 1990; Butler *et al.*, 1993a) a further discussion of the mechanisms of oxygen transport by blood to the tissues is in order.

Although a small amount of the oxygen in blood is transported in solution in plasma and intracellular fluid, the vast majority is carried reversibly bound to haemoglobin. Haemoglobin is a complex molecule consisting of four protein chains to each of which is attached a haem group (an iron atom surrounded by a porphyrin ring), the latter being the oxygen binding site. A carrier system of this type is necessary since the amount of oxygen which could be carried in solution is many fold less than is required to sustain adequate oxygen delivery to the tissues at rest without an impossibly high cardiac output. The shape of the haemoglobin molecule (the so-called quaternary structure) is an important determinant of oxygen affinity and alterations in this structure brought about by factors such as pH, temperature and carbon dioxide are the mechanism by which these variables affect

haemoglobin oxygen affinity (see below).

The relationship of the percentage saturation ( $S_{O_2}$ ) of haemoglobin in blood to the partial pressure of the oxygen in solution in it is described by the so-called oxyhaemoglobin dissociation curve (Figure 7.18). This curve is sigmoid in shape due to the interaction between the four oxygen binding sites on the haemoglobin molecule whereby the binding of an oxygen molecule to each site influences binding on the next site.

This relationship between oxygen tension and saturation has several biological advantages. Since at higher oxygen tensions the curve is relatively flat, a slight reduction in  $P_{aO_2}$  will have a negligible effect on haemoglobin saturation and hence on amount of oxygen carried. In the exercising horse, the effect of exercise-induced arterial hypoxaemia on oxygen saturation is considerably less than would be the case were the relationship between haemoglobin saturation and oxygen tension a linear one. In this study, during C12+3 mean  $S_{aO_2}$  fell to 78.4% ( $\pm 2.7$ ) from a resting mean of 97.5% ( $\pm 0.1$ ), associated with a fall in mean  $P_{aO_2}$  from 94.1 mm Hg ( $\pm 1.7$ ) to 60.2 mm Hg ( $\pm 3.0$ ). If the oxyhaemoglobin dissociation curve were linear,  $S_{aO_2}$  would have fallen by almost twice as much to around 62%.

In the horse, exercise or excitement causes a sympathetically-driven increase in packed cell volume and hence in blood haemoglobin concentration due to release into the circulation of erythrocytes stored in the spleen. In this study, during C12-3, this resulted in an increase in oxygen-carrying capacity of 41% (page 139 and Figure 7.10). As a result, arterial oxygen content remains fairly constant as exercise level increases, even in the presence of arterial

hypoxaemia, mean values for  $C_{aO_2}$  staying between 12.08 mmol/l and 12.22 mmol/l during C8, C10 and C12, over the course of which mean  $P_{aO_2}$  dropped from 84.3 mm Hg to 66.7 mm Hg. Even during C12+3, when mean  $P_{aO_2}$  was as low as 60.2 mm Hg, mean  $C_{aO_2}$  only fell to 11.37 mmol/l and was still significantly higher than the resting mean value of 9.88 mmol/l (see Figure 7.11).

At lower oxygen tensions, the 'steep' portion of the oxyhaemoglobin dissociation curve implies that oxygen release to the peripheral tissues is facilitated as there is a large change in haemoglobin saturation for a small change in oxygen tension.

It was mentioned above that various factors may change the quaternary structure of the haemoglobin molecule thereby influencing its affinity for oxygen and this alters the shape of the oxyhaemoglobin dissociation curve. A decrease in blood pH, an increase in temperature and an increase in  $P_{CO_2}$  all cause a shift of the curve to the right (Figure 7.18), i.e. at a given  $SO_2$  the  $PO_2$  will be higher (the effect of pH in this way is known as the 'Bohr effect'). Since pH, temperature and  $P_{CO_2}$  in the peripheral exercising muscle all show alterations in these directions, there is a reduced affinity of haemoglobin for oxygen at this site which facilitates release of oxygen into the tissues, the increased  $PO_2$  in muscle capillary blood at a given saturation providing an increase in blood-tissue  $PO_2$  difference and hence an increased diffusion partial pressure gradient.

Although it has been suggested that the exercising horse tolerates a respiratory acidosis because it would be too costly in terms of ventilatory workload to maintain normocapnia (Bayly *et al.*, 1989) a further factor may be that a degree of respiratory acidosis will tend

to increase oxygen diffusion into the tissues. Furthermore, in man moderate respiratory acidosis increases cerebral blood flow and cardiac output which would also tend to increase venous and hence tissue  $P_{O_2}$  further (Nunn, 1987). Thus there may be advantages in terms of tissue oxygen delivery in allowing the development of a degree of respiratory acidosis during exercise.

The shift to the right of the oxyhaemoglobin dissociation curve in the muscle capillary beds also has implications for carbon dioxide transport. The majority of the carbon dioxide in the blood is transported as bicarbonate, but smaller amounts are carried in physical solution or in the carbamino form bound to amino groups. Most of the carbon dioxide in the carbamino form is carried by haemoglobin (with a much smaller amount carried in this form on plasma proteins). Carbamino transport of carbon dioxide by haemoglobin is affected by oxygen saturation, with (at a constant  $P_{CO_2}$ ) more being carried at lower saturations. In addition, reduction of haemoglobin results in an increased buffering capacity allowing increased carbonic acid dissociation and hence an increase in carbon dioxide transport as bicarbonate. The so-called 'Haldane effect', the difference in carbon dioxide carriage (at a constant  $P_{CO_2}$ ) between oxygenated and reduced blood is due to these factors, with the greater part being due to increased carbamino carriage.

As reported previously (Bayly *et al.*, 1983; Thornton *et al.*, 1983; Bayly *et al.*, 1987a, Evans and Rose, 1988a and 1988b; Littlejohn and Snow, 1988; Anderson *et al.*, 1989; Bayly *et al.*, 1989; Wagner *et al.*, 1989; Art *et al.*, 1990; Butler *et al.*, 1993a), strenuous exercise was found to be accompanied by arterial hypoxaemia (associated with an increase in  $P_{(ET-A)}O_2$  and hypercapnia). It is generally considered that the arterial hypoxaemia results

in the main, from diffusion limitation at the pulmonary blood gas barrier, with a smaller contribution from a relative hypoventilation (Anderson *et al.*, 1989; Wagner *et al.*, 1989). Evidence for the former cause comes from the progressive increase in  $P_{(ET-a)}O_2$ , which in the absence of an increase in  $P_{(a-ET)}CO_2$  is unlikely to be associated with worsening of ventilation:perfusion matching (Anderson *et al.*, 1989). The arterial hypercapnia indicates that hypoventilation also occurs. Multiple inert gas elimination studies have supported this interpretation of the causes of the arterial hypoxaemia of exercise (Wagner *et al.*, 1989).

It has been suggested that tolerance of arterial hypercapnia during exercise may be a stratagem for avoidance of an uneconomically high ventilatory work load (Bayly *et al.*, 1989, see above). Another feature of equine respiration during canter which may have an energy sparing role is the entrainment of respiration and locomotion which has been extensively reported previously (Cook, 1965; Specht, 1965; Attenburrow, 1971, 1982 and 1983; Attenburrow and Flack, 1974; Hörnicke *et al.*, 1974b; Hörnicke and Meixner, 1977; Bramble and Carrier, 1983; Hörnicke, Meixner and Pollman, 1983; Anderson *et al.*, 1990; Frevert *et al.*, 1990; Young *et al.*, 1992).

Butler *et al.*, (1993a) suggested that the relative hypoventilation of exercise may be contributed to by an unusually high  $V_d/V_t$  ratio since in that study  $V_d/V_t$  during exercise remained above resting values. In the present study, however,  $V_d/V_t$  was lower than the reported resting value of Butler *et al.* during C10, C12 and C12-3. Although  $V_d$  was slightly higher during exercise, its increase with work load was modest compared to that of  $V_A$ .



Butler *et al.* (1993a) pointed out that the ultrasound flow transducer system produced a higher  $\dot{V}_e$  related to  $\dot{V}_{O_2}$  than earlier studies involving systems using valves. In the present study, the values for  $\dot{V}_e$  were similarly higher than would be expected using valve system at the same workload.

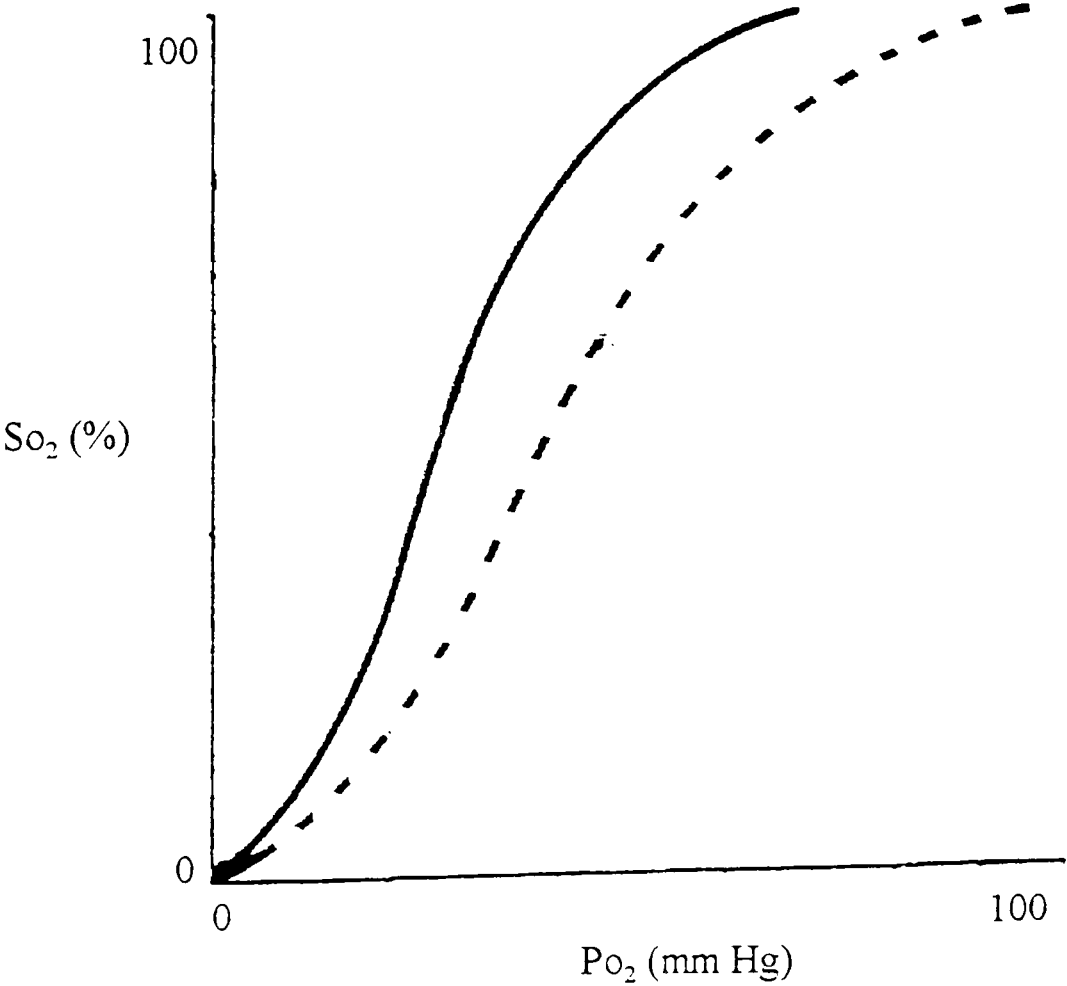
It is interesting that PEF appeared to level off at the highest workloads in three out of five horses for whom full data was available. Although it was once thought that the healthy respiratory system was unlikely to limit performance in man or the horse (Gillespie, 1974), more recently it has been suggested that in trained athletes ventilation may be limiting both in man (Dempsey, 1985; Whipp, 1992) and the horse (Art and Lekeux, 1989a and 1989b; Art *et al.*, 1990; Butler *et al.*, 1993a). In this context, it is also noteworthy that whilst the increase in oxygen carrying capacity of the blood during exercise (conferred by the increase in PCV and hence in blood haemoglobin concentration due to sympathetically-mediated splenic contraction) more than compensates for the effects of arterial hypoxaemia at the lower levels of exercise, at the highest workload (C12+3)  $C_{aO_2}$  was lower than in the other three less strenuous canters. It may be that at high workloads arterial desaturation becomes a significant factor in the limitation of oxygen transport.

Although no plateau of  $\dot{V}_{O_2}$  occurred, the highest values for it were similar to values for  $\dot{V}_{O_{2max}}$  reported by other workers (Evans and Rose, 1988b; Butler *et al.*, 1991; Knight, Sinha and Rose, 1991) and higher than some values reported by other workers (Evans, 1987; Rose *et al.*, 1988).

The respiratory responses of the horses in this study were similar to those reported

previously, in particular when compared to studies using the same measuring methods. During recovery from the canter periods a similar hyperventilatory response was observed as has been reported previously (Butler *et al.*, 1993a). The data obtained from these horses will be used later for comparison with results from referred clinical cases (Chapter Eleven).

**Figure 7.18: Diagram of oxyhaemoglobin dissociation curve relating blood haemoglobin saturation to oxygen tension. Curved line demonstrates shift to the right (see text for explanation).**



**CHAPTER EIGHT - THE EFFECT OF TRAINING ON THE RESPIRATORY  
RESPONSE TO EXERCISE**

The previous chapter discussed the respiratory response to exercise in six fully-trained Thoroughbred horses. This chapter is concerned with the effect of training on the respiratory response to exercise in six untrained Thoroughbred horses.

**Summary of Methods**

Eight Thoroughbred horses which had been turned out and not exercised for at least five months were trained in a conventional race training<sup>1</sup> regime over a period of sixteen weeks. Exercise tests were performed before training and after six, eleven and sixteen weeks of training (designated ET0, ET6, ET11 and ET16 respectively). The tests carried out during training were timed to coincide with the end of each stage of training (walking and trotting only, cantering and galloping).

The exercise test consisted of a warm up period (at walk and trot) followed by three, two minute periods of canter at 8, 10 and 12 m.s<sup>-1</sup> (again, designated C8, C10 and C12

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<sup>1</sup> i.e. ridden exercise for approximately one to one and a half hours per day on six days per week. The horses walk and trot on the way to and from the training gallops where one, or usually two, gallops of 1200 to 1800m duration are performed. A typical regime would be:

Monday:	walking and trotting only
Tuesday:	'canter' (i.e. moderate speed gallop)
Wednesday:	'work day' (fast galloping)
Thursday:	canter
Friday:	canter
Saturday:	'work day'
Sunday:	rest day

respectively), separated by ten minute walks. In ET16, the test was continued to include a further canter at 12 m.s<sup>-1</sup> on a three degree incline (C12+3) since these final tests also acted as the initial pre-challenge tests for the third viral challenge study. For the purposes of this study, data are presented for only the C8, C10 and C12 canters, i.e. those which were performed in all tests during the training period. The data from C12+3 are used for the V3 investigation of the effects of influenza challenge on respiratory function, which is reported later in Chapter Ten. For further details of the horses, training regime, exercise test protocol and method of data collection, see Chapter Three.

Figures 8.1 to 8.18 present the results of all tests, data are plotted as means  $\pm$  SEM. A paired, two tailed Student's *t* test was used to compare the pre-training results (ET0) with those from the end of training (ET16), differences were regarded as significant at the 0.05 level of probability.

## Results

Two horses dropped out during the study, one slipped and fell during ridden exercise (between ET0 and ET6), sustaining a cortical fracture of the left tibia. The other developed superficial digital flexor tendinitis in the left foreleg during ridden exercise shortly after ET6. Data are presented from the six animals that completed the study.

During training all horses lost weight, the mean body weight of the group being 496kg before training and 478kg at the end of training.

$\dot{V}_e$  and  $f_b$  during exercise were not significantly altered by training (Figures 8.1 and 8.2).

Since respiratory-locomotory synchrony during canter was maintained, stride rate must also have been unaltered at this gait. Expired tidal volume ( $V_t$ ) was significantly higher following training during trot but not during canter (Figure 8.3).

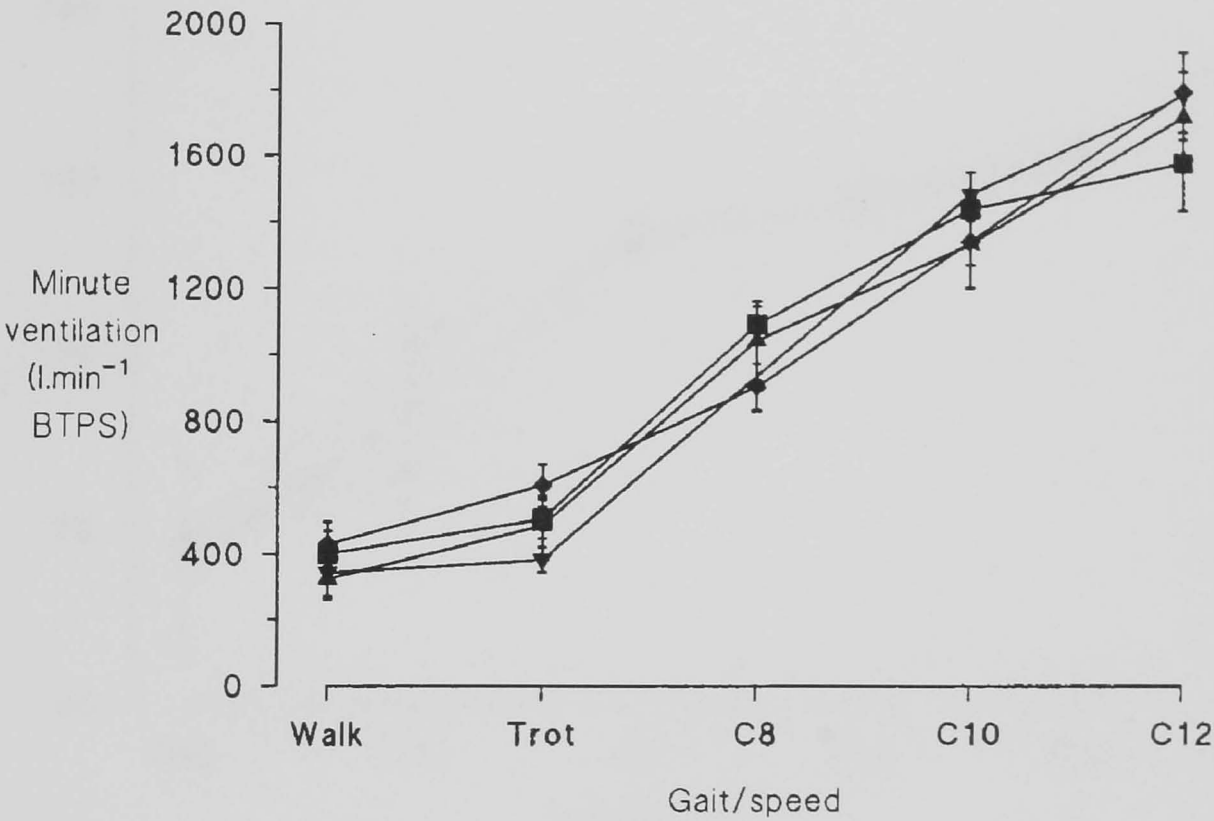
Peak inspiratory flow rate (PIF) was significantly lower following training during C8 and C10, but not at the highest canter speed (Figure 8.4). Peak expiratory flow rate (PEF) was significantly lower following training only during C8 (Figure 8.5). During C10, PEF was also lower after training but the difference did not achieve significance ( $p=0.525$ ).

Inspiratory time ( $t_i$ ) and expiratory time ( $t_e$ ) were similar at most exercise levels before and after training, the only significant alteration in these parameters being at C12 when  $t_i$  was higher before training than after (Figure 8.6). Although not significantly different from post-training values,  $t_e$  was consistently lowest during ET0 and this was reflected in  $t_i/t_e$  (Figure 8.7), the highest values for which also occurred before training, although this parameter was only significantly higher at this stage during trot (during C12 the difference approached significance,  $p=0.0544$ ).

$V_{dp/hys}/V_t$  was not significantly altered by training.

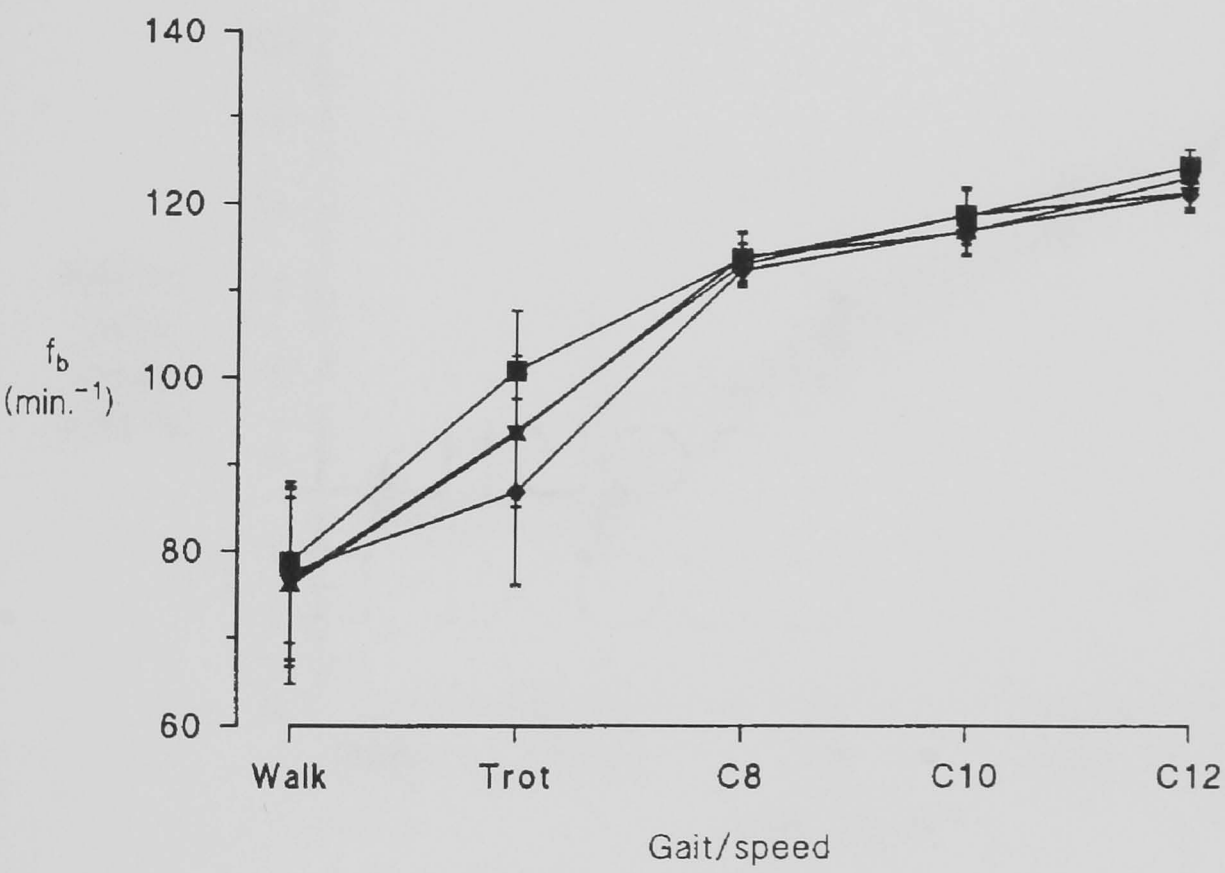
Arterial-end tidal  $P_{CO_2}$  difference ( $P_{a-ET}CO_2$ ) also showed no significant modification following training, suggesting no alteration in alveolar dead space.

**Figure 8.1: Changes in minute ventilation during exercise at different stages of training**

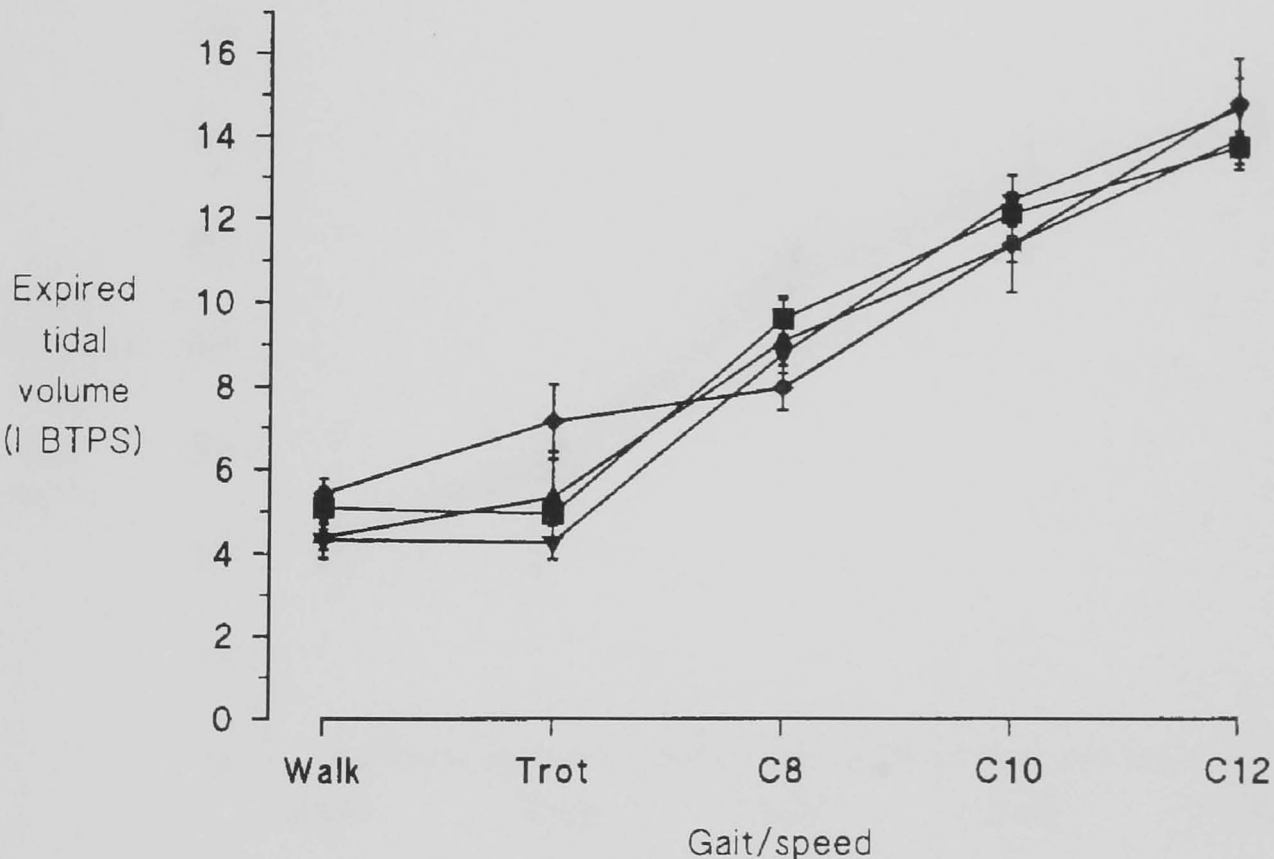


Key for Figures 9.1–9.18: ET0 ■ ET6 ▲ ET11 ▼ ET16 ◆

**Figure 8.2: Changes in respiratory frequency during exercise at different stages of training**

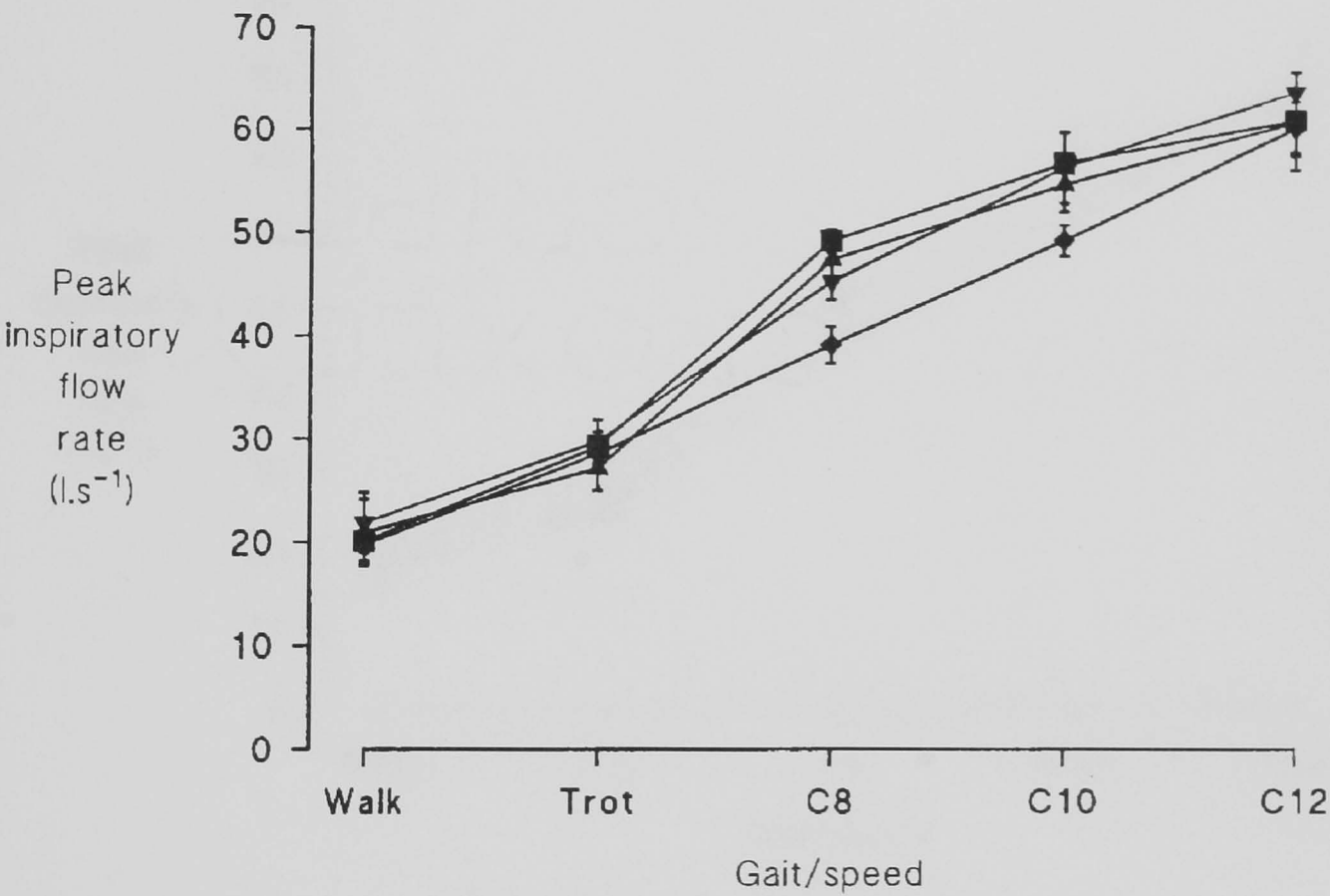


**Figure 8.3: Changes in expired tidal volume during exercise at different stages of training**

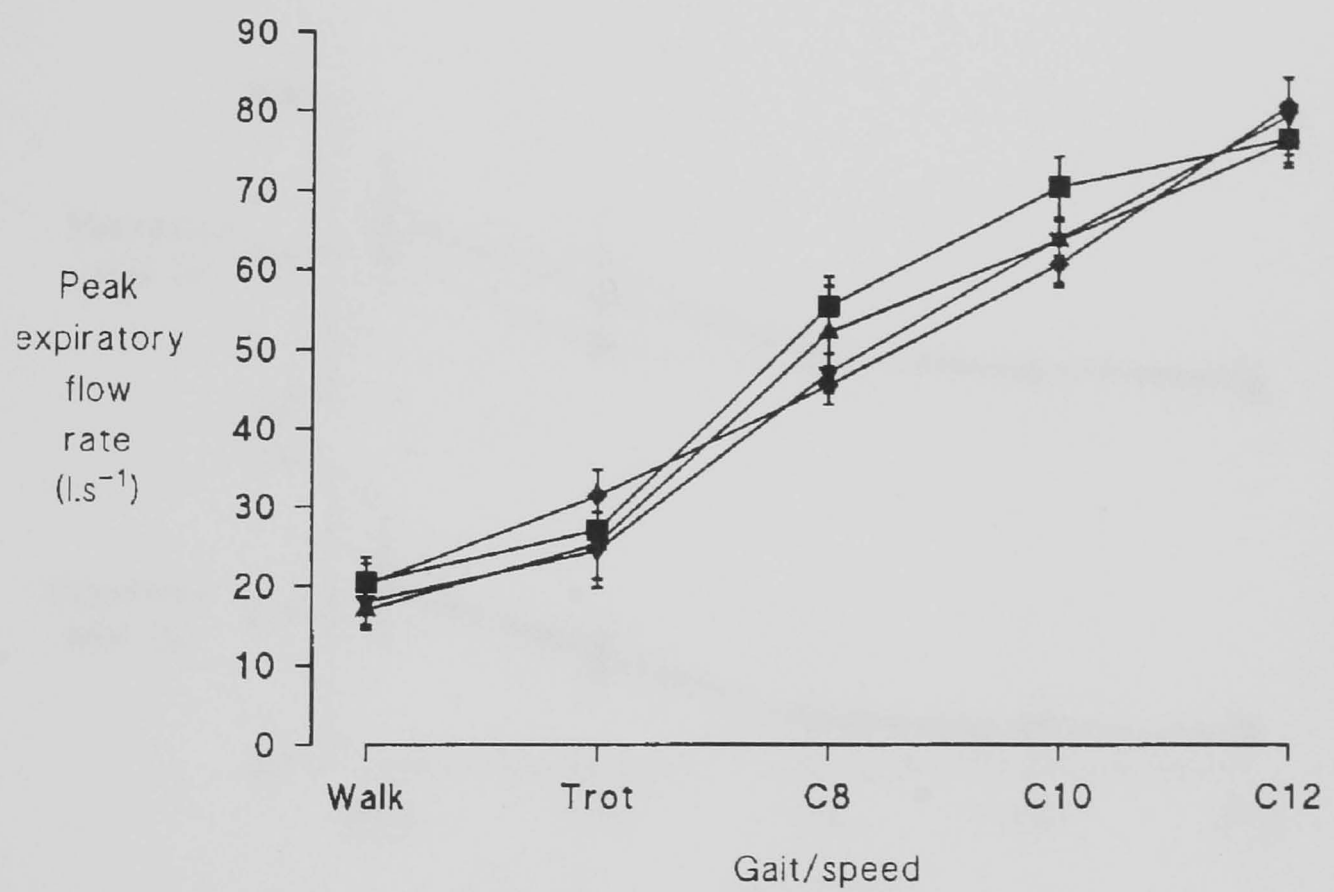




**Figure 8.4: Changes in peak inspiratory flow rate during exercise at different stages of training**



**Figure 8.5: Changes in peak expiratory flow rate during exercise at different stages of training**



**Figure 8.6: Changes in inspriatory and expiratory times during exercise at different stages of training**

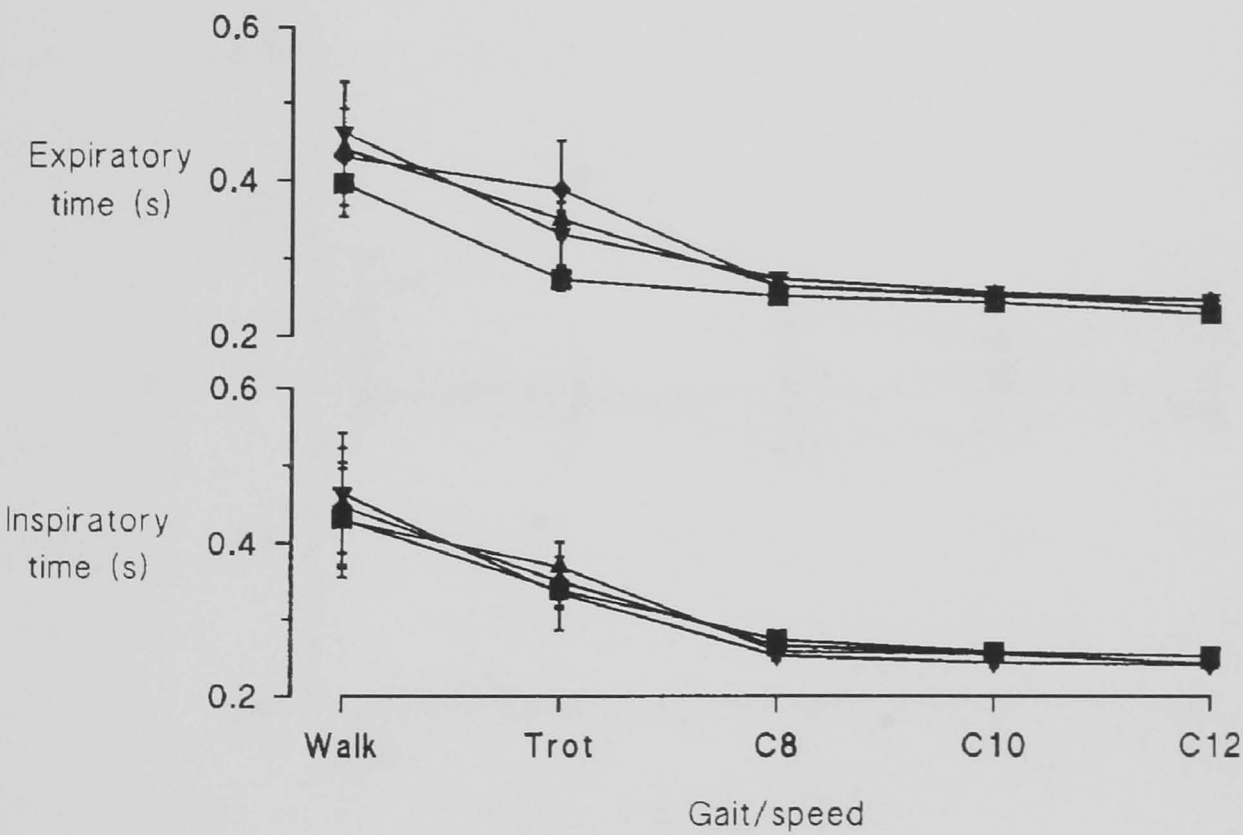
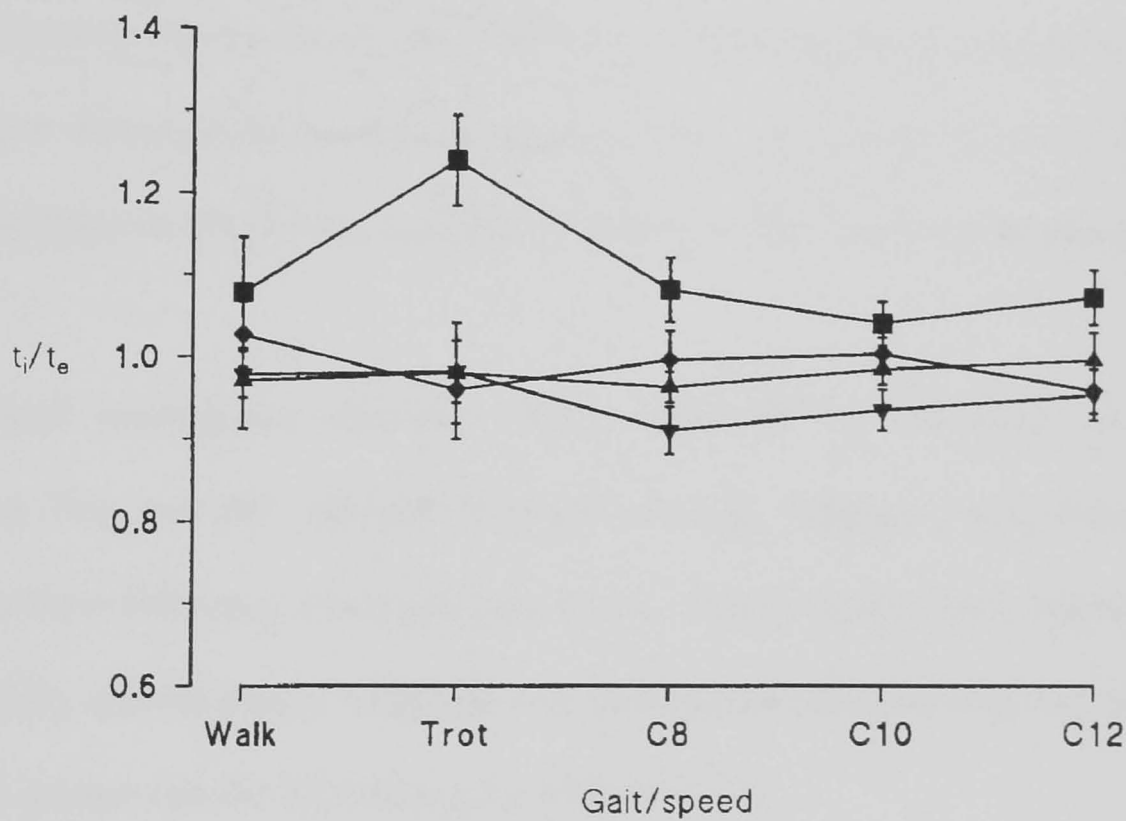


Figure 8.7: Changes in inspiratory/expiratory time ratio during exercise at different stages of training



Mean mixed venous temperature ( $t_{\bar{v}}$ ) was 0.4-0.6°C higher at rest for ET0 than for the subsequent studies. During exercise,  $t_{\bar{v}}$  remained highest for ET0 and was significantly greater than following training at all speeds except C12 (Figure 8.8).

While resting and walking  $P_{aO_2}$  was similar at all stages of training, during trot and canter it decreased significantly with training (Figure 8.9).  $P_{\bar{v}O_2}$ , alveolar oxygen tension<sup>2</sup> ( $P_{AO_2}$ ), and  $P_{\bar{v}CO_2}$  were unchanged by training (Figures 8.9 and 8.10).  $P_{aCO_2}$  was only significantly different following training during trot, when it was higher compared with initial values (Figure 8.10). The end tidal-arterial  $PO_2$  difference ( $P_{(ET-a)O_2}$ ) showed a trend to increase with training (Figure 8.11) and was significantly greater for the trained horses during C12.

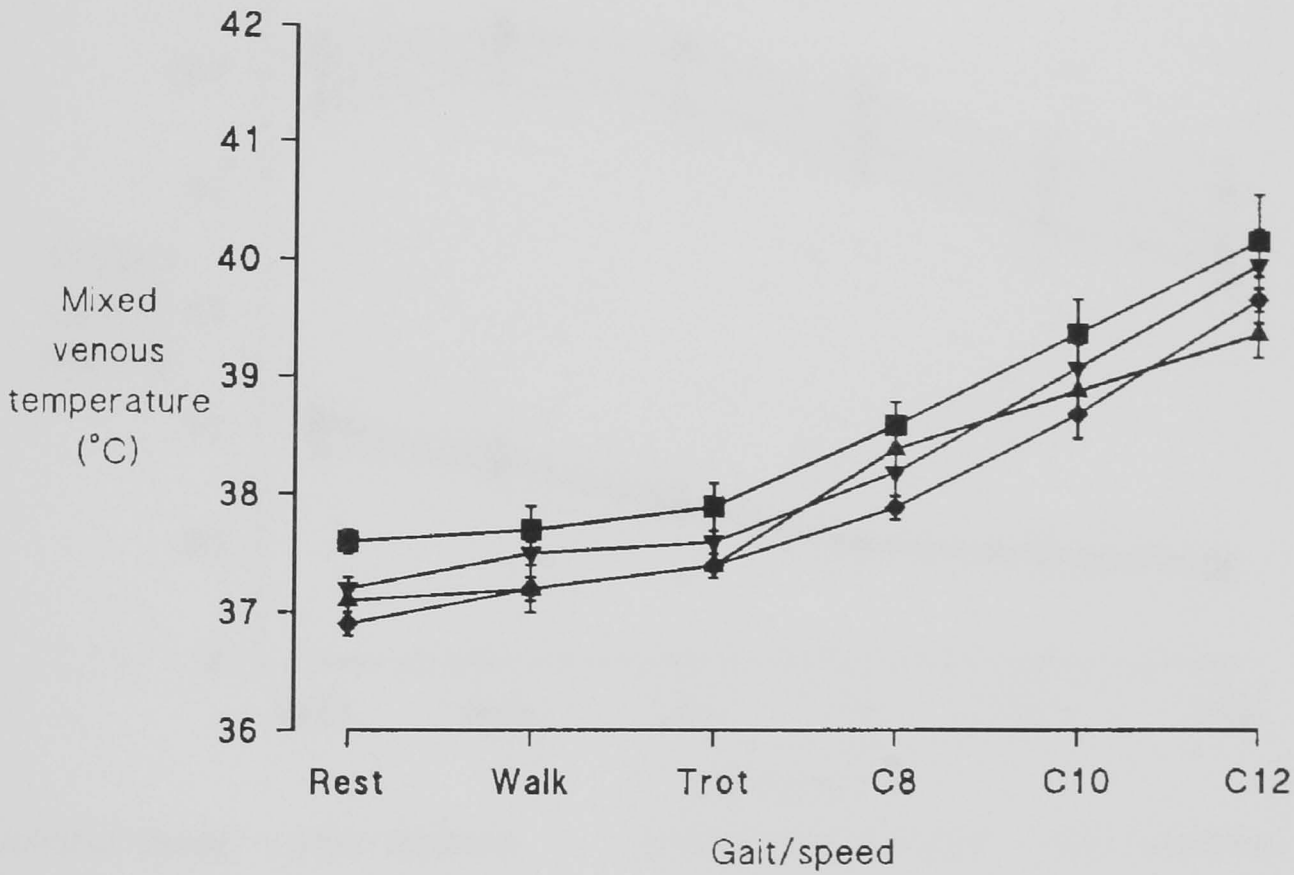
Arterial blood haemoglobin saturation ( $S_{aO_2}$ ), corrected for differences in blood temperature,  $P_{CO_2}$  and  $pH$ <sup>3</sup>, changed little with training, although during trot it was significantly lower following training (Figure 8.12a). Mixed venous blood haemoglobin saturation ( $S_{\bar{v}O_2}$ ) showed a trend to be higher at all points following training but was only significantly greater than for ET0 during C8 (Figure 8.12b).

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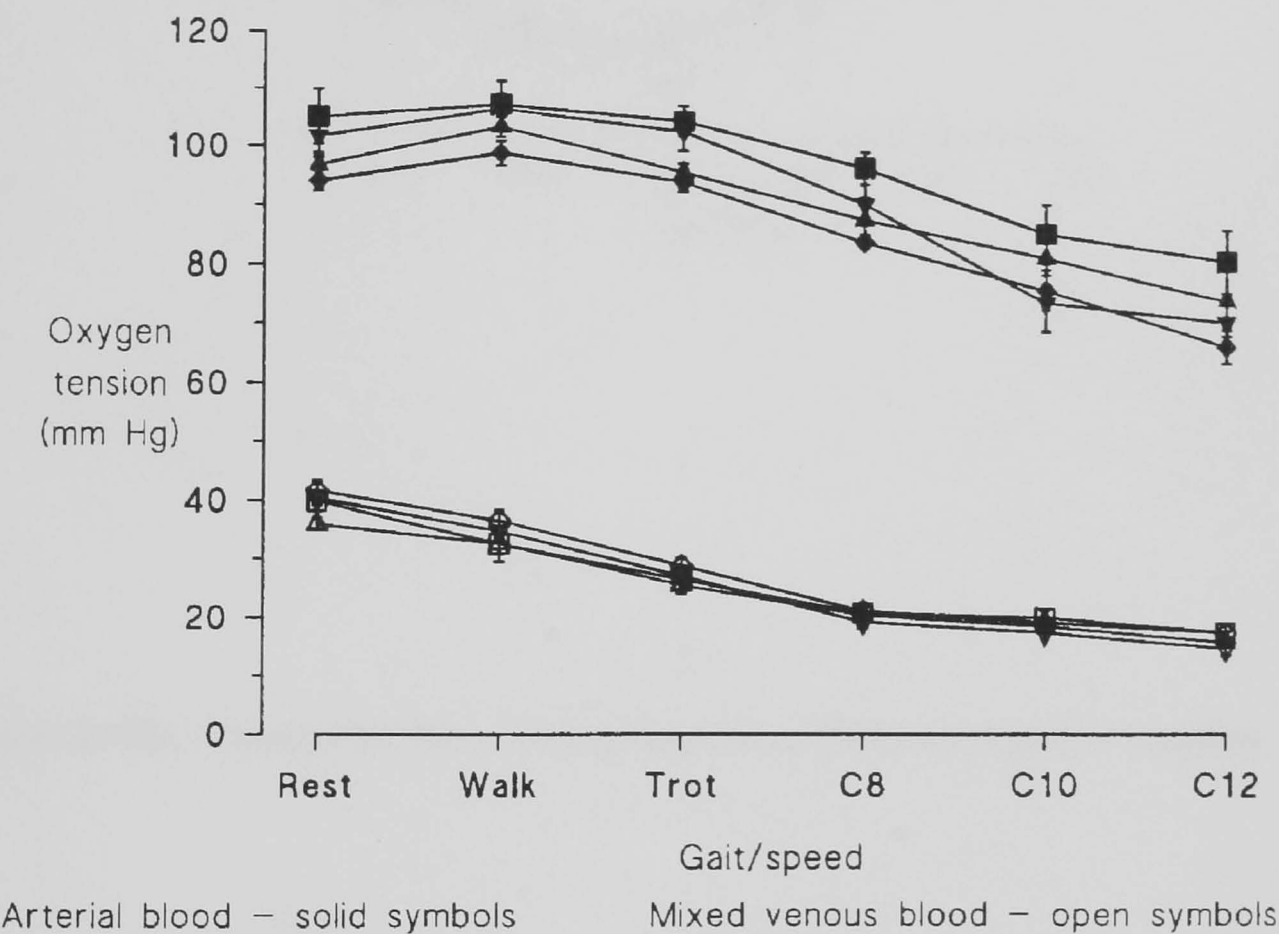
<sup>2</sup>Since this parameter was derived using the alveolar gas equation, it could only be calculated when  $\dot{V}O_2$  and hence respiratory exchange ratio, could be obtained. Within these confines there did not appear to be any change in  $P_{AO_2}$ .

<sup>3</sup>Using an algorithm for equine blood modified by Anderson and Smale (1990) from that of Kelman (1966) for human blood.

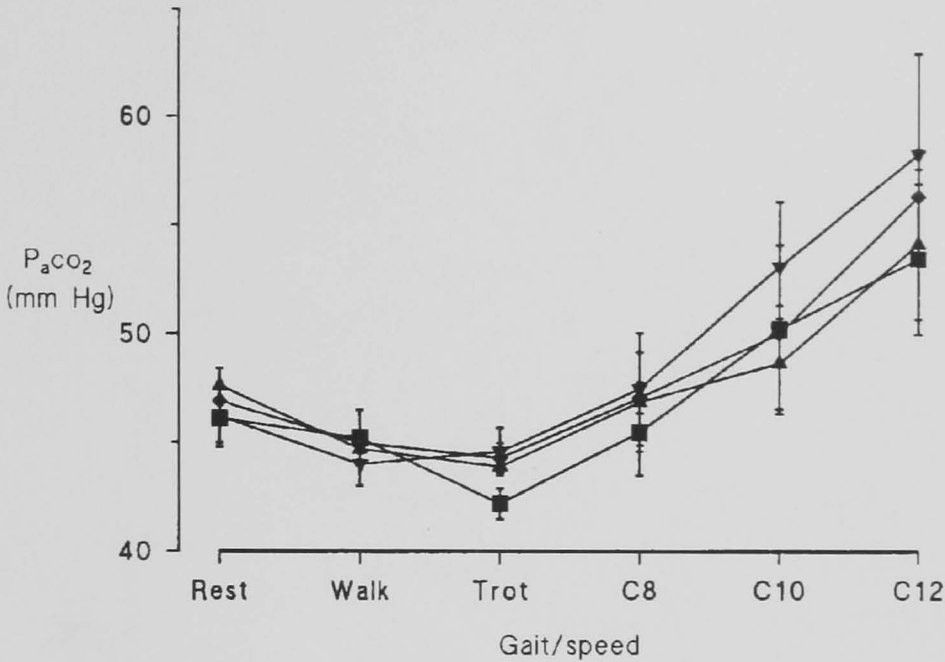
**Figure 8.8: Changes in mixed venous temperature during exercise at different stages of training**



**Figure 8.9: Changes in blood oxygen tensions during exercise at different stages of training**



**Figure 8.10a: Changes in  $P_a\text{CO}_2$  during exercise at different stages of training**



**Figure 8.10b: Changes in  $P_v\text{CO}_2$  during exercise at different stages of training**

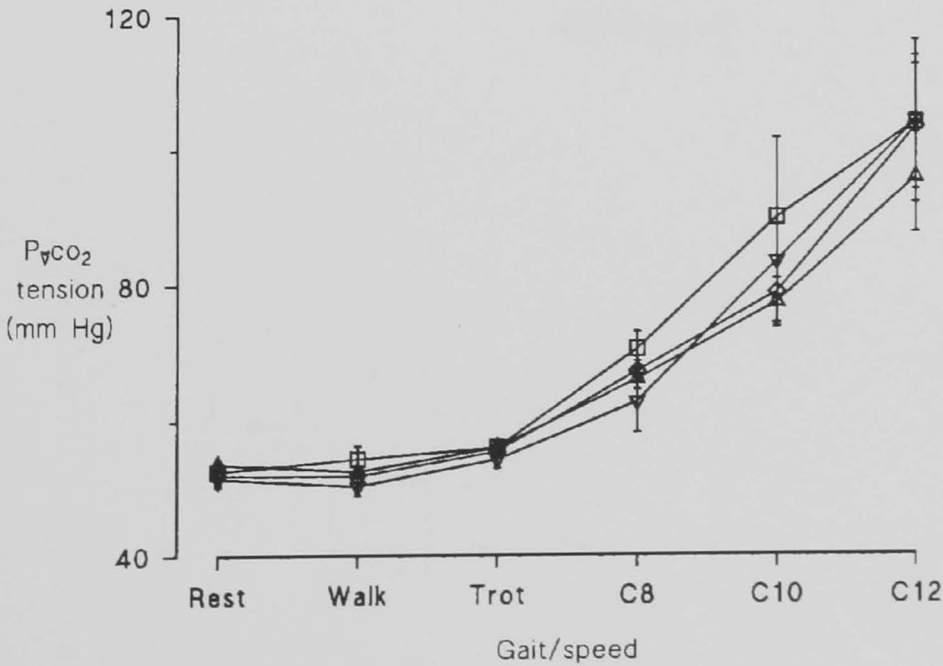
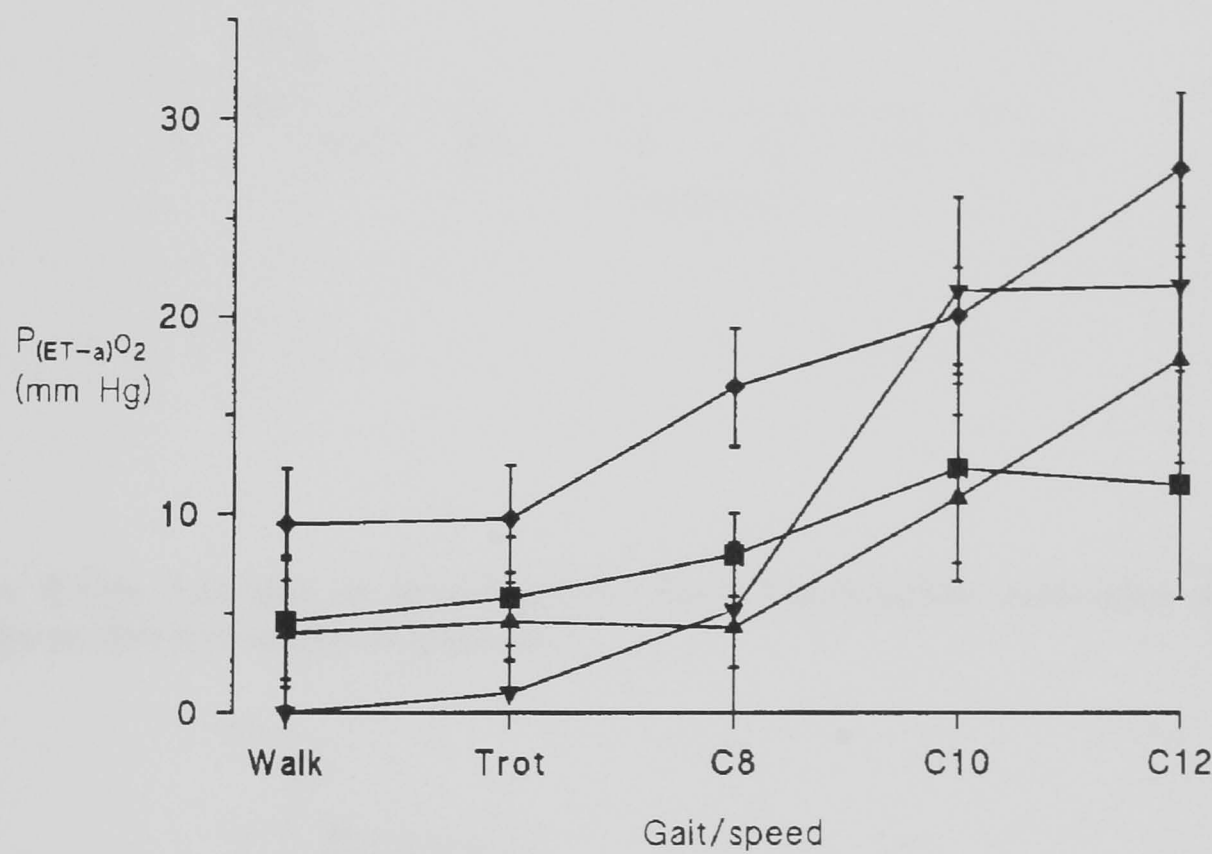
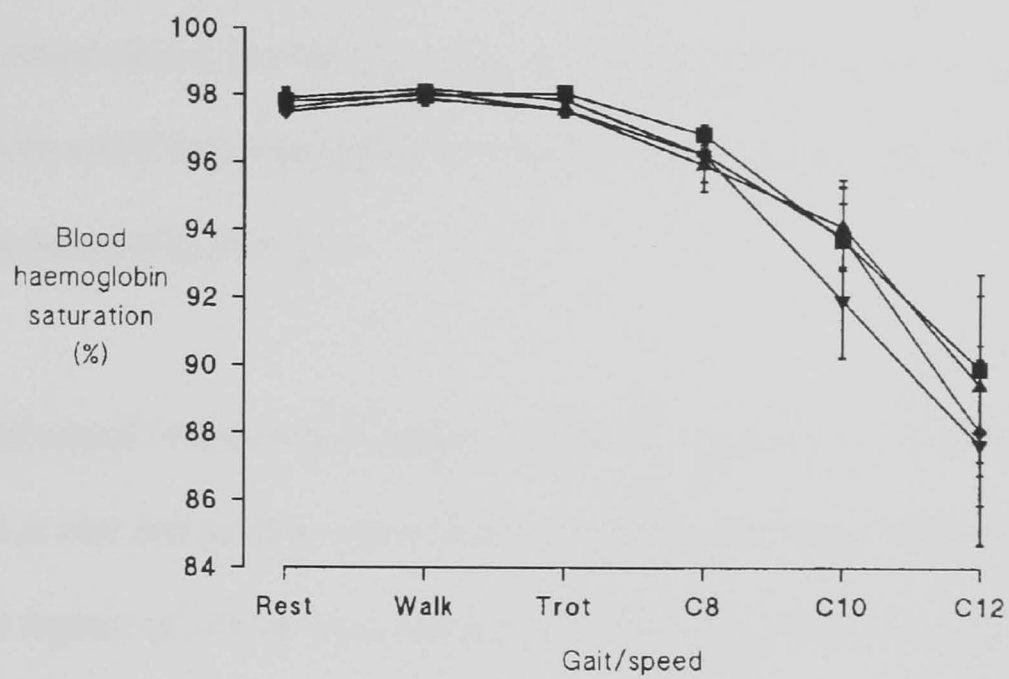




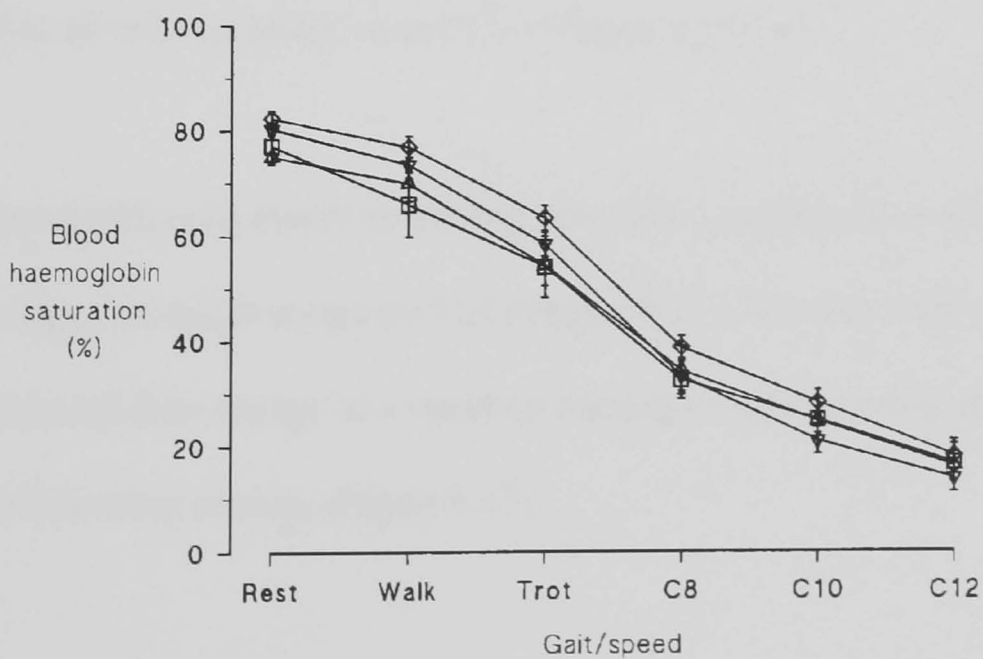
Figure 8.11: Changes in  $P_{(ET-a)O_2}$  during exercise at different stages of training



**Figure 8.12a: Changes in arterial blood haemoglobin saturation during exercise at different stages of training**



**Figure 8.12b: Changes in mixed venous blood haemoglobin saturation during exercise at different stages of training**



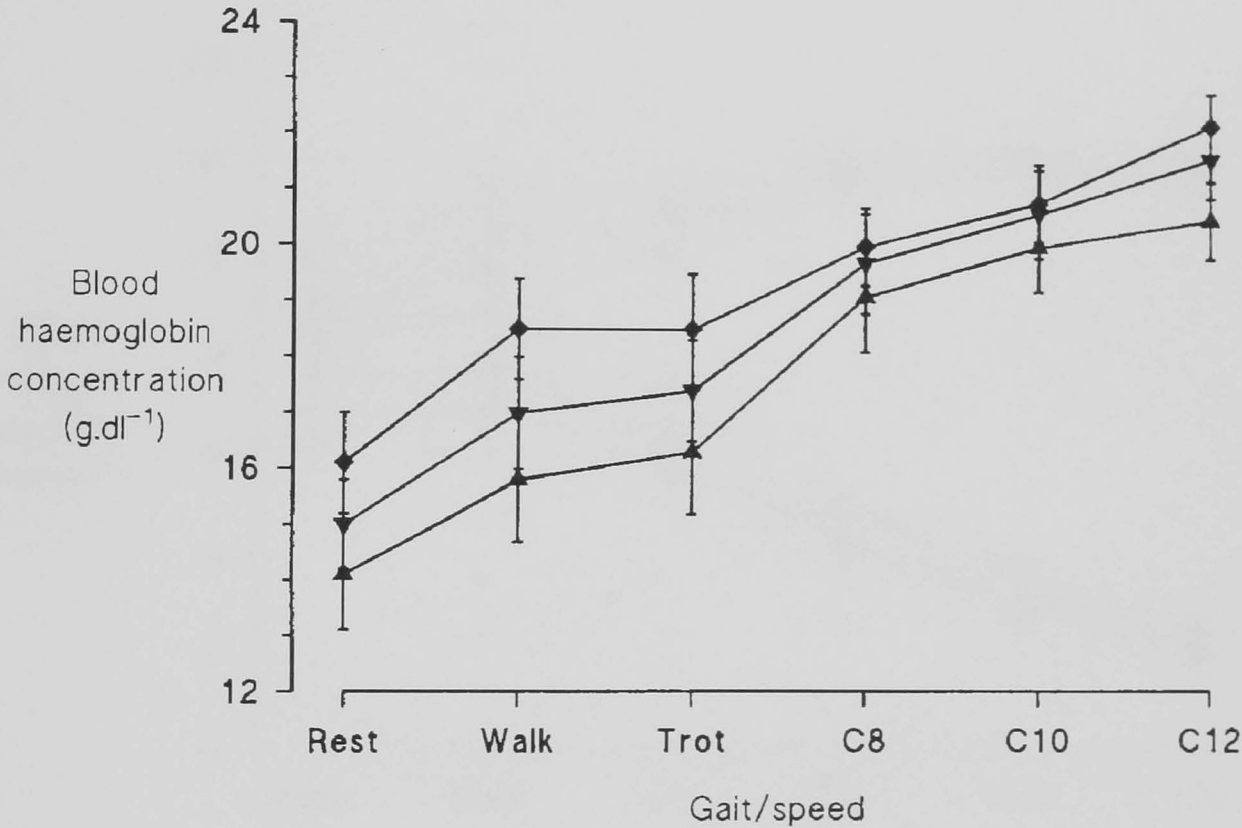
Unfortunately, it was not possible to measure blood haemoglobin concentration in ET0 as the samples were damaged prior to analysis, so for this parameter and the calculated oxygen content data it has been possible to compare the final three exercise tests only. Between ET6 and ET16, haemoglobin concentration increased significantly at rest and at all exercise levels (Figure 8.13).

Arterial and mixed venous blood oxygen content ( $C_{aO_2}$  and  $C_{\bar{v}O_2}$ ) increased between ET6 and ET16 at rest and at all exercise levels, the difference being significant at all speeds except the highest (C12) for  $C_{aO_2}$  and at rest, walk and trot for  $C_{\bar{v}O_2}$  (Figure 8.14). The arterial-mixed venous blood oxygen content difference ( $C_{a-\bar{v}O_2}$ ) was hardly changed between ET6 and ET16, although it was significantly lower after training during trot (Figure 8.15).

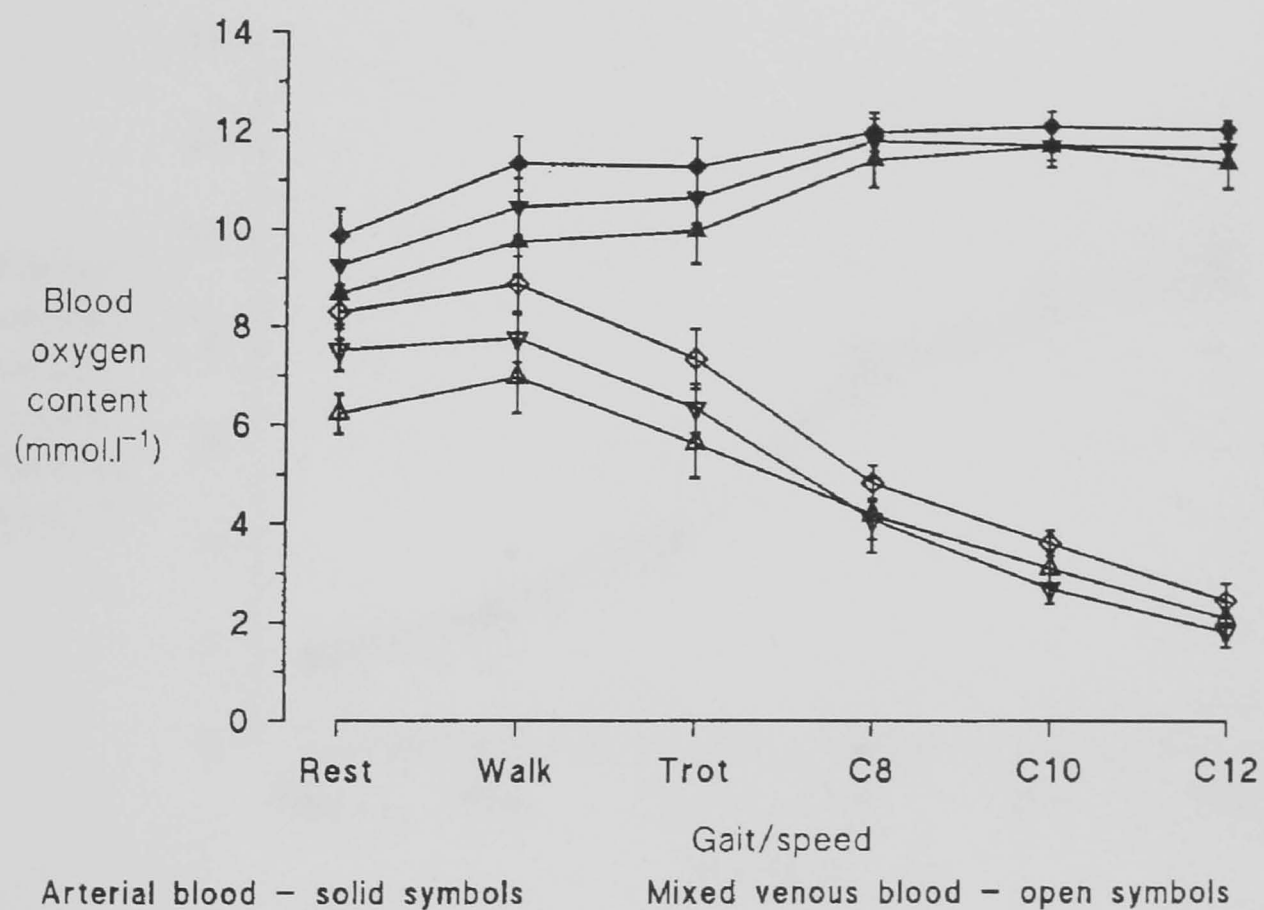
Arterial and mixed venous blood pH increased with training, the increase being significant at rest and at all exercise levels except C12 (Figure 8.16).

Although not significantly altered by training, end tidal oxygen tension ( $P_{ET}O_2$ ) was highest before training at all speeds except for C12 (Figure 8.17). End tidal carbon dioxide tension ( $P_{ET}CO_2$ ) showed little change as a result of training except for being significantly higher during trot following training (Figure 8.17).

**Figure 8.13: Changes in blood haemoglobin concentration during exercise at different stages of training**



**Figure 8.14: Changes in blood oxygen content during exercise at different stages of training**



**Figure 8.15: Changes in arterial-mixed venous oxygen content difference during exercise at different stages of training**

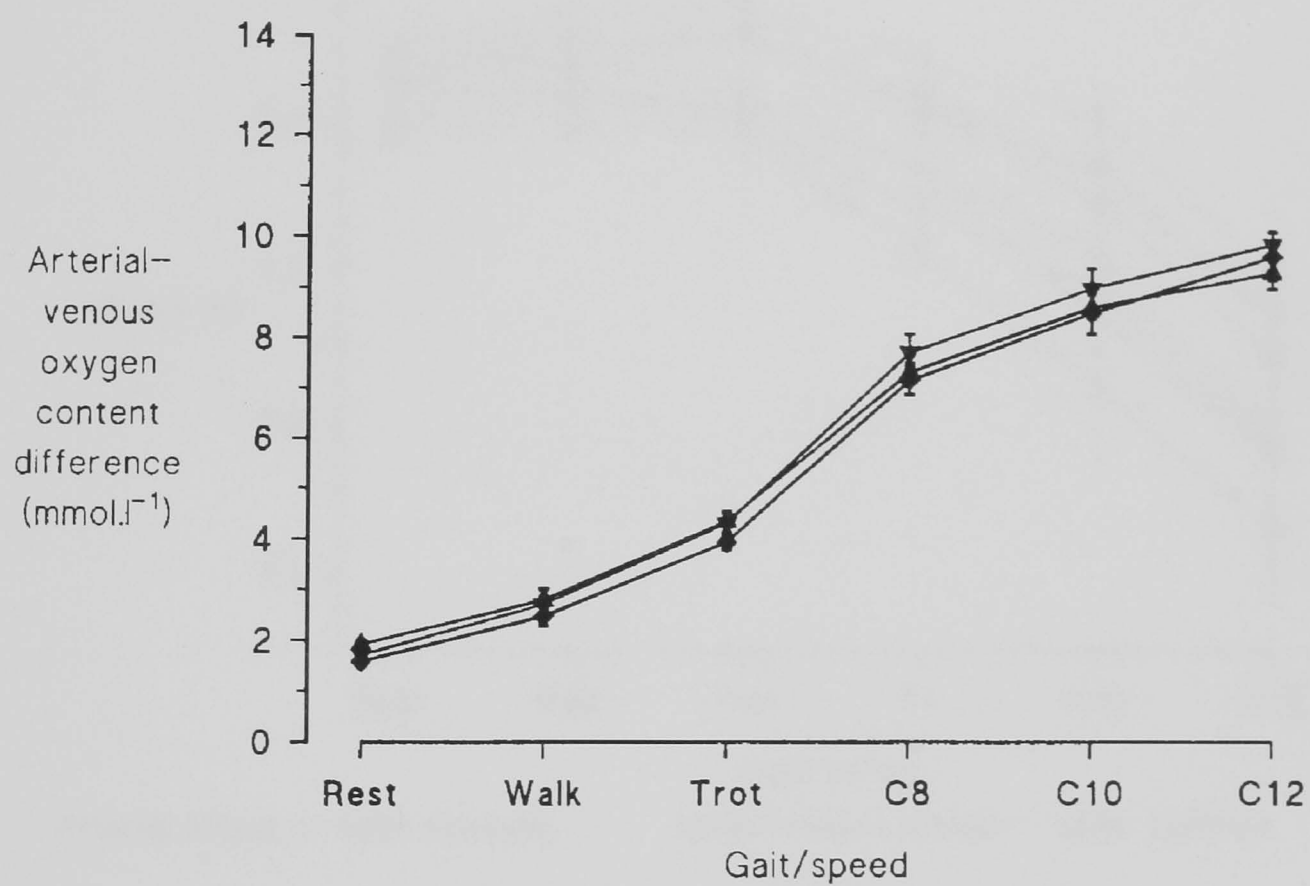


Figure 8.16: Changes in blood pH during exercise at different stages of training

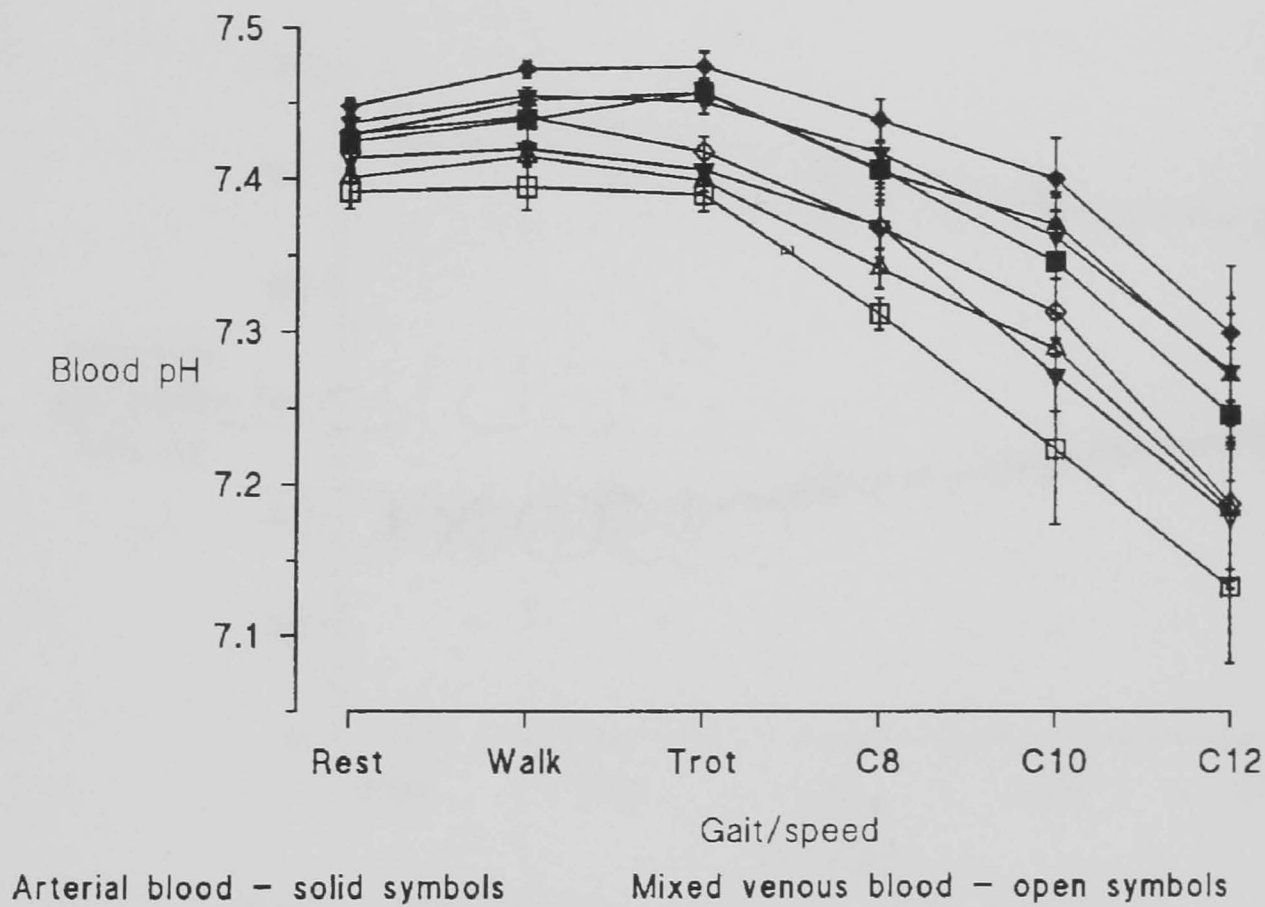
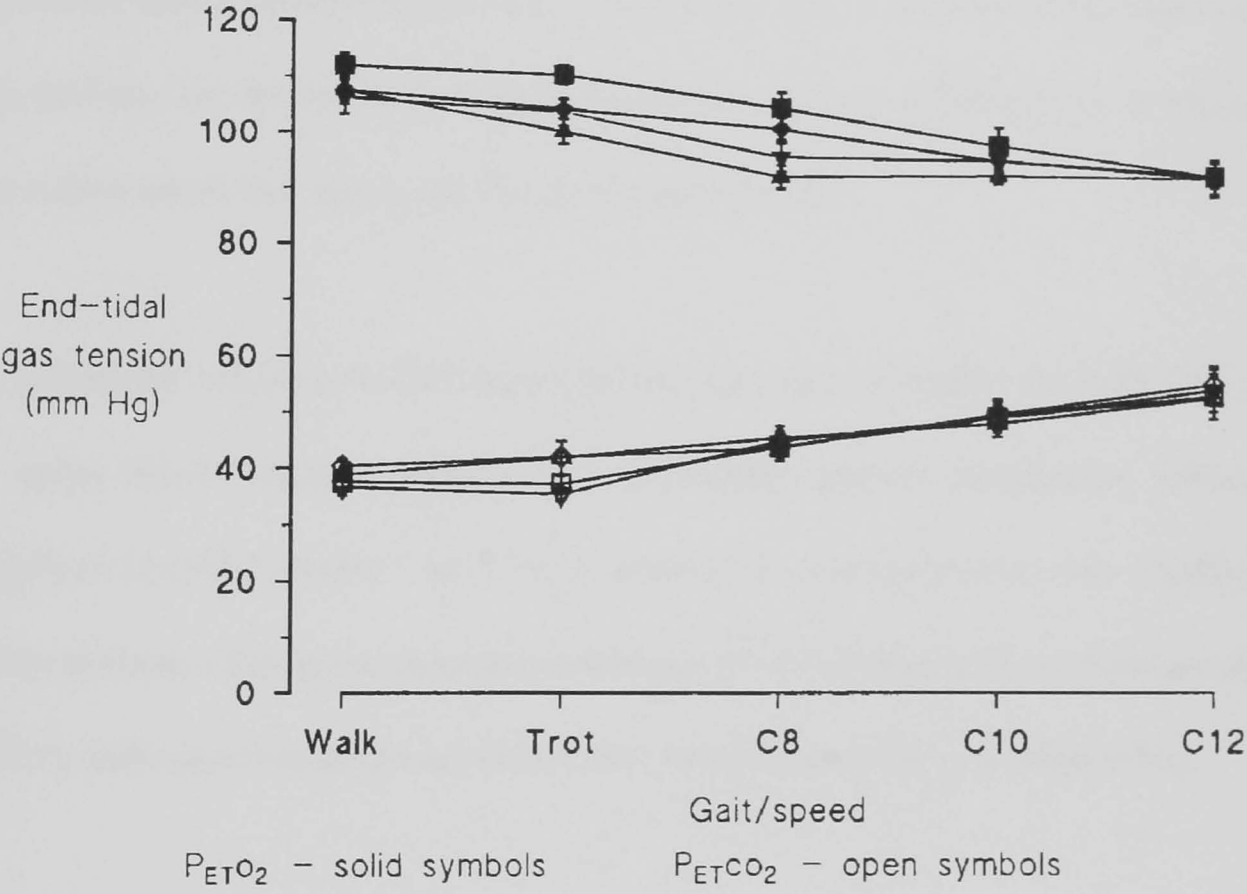


Figure 8.17: Changes in end-tidal gas tensions during exercise at different stages of training





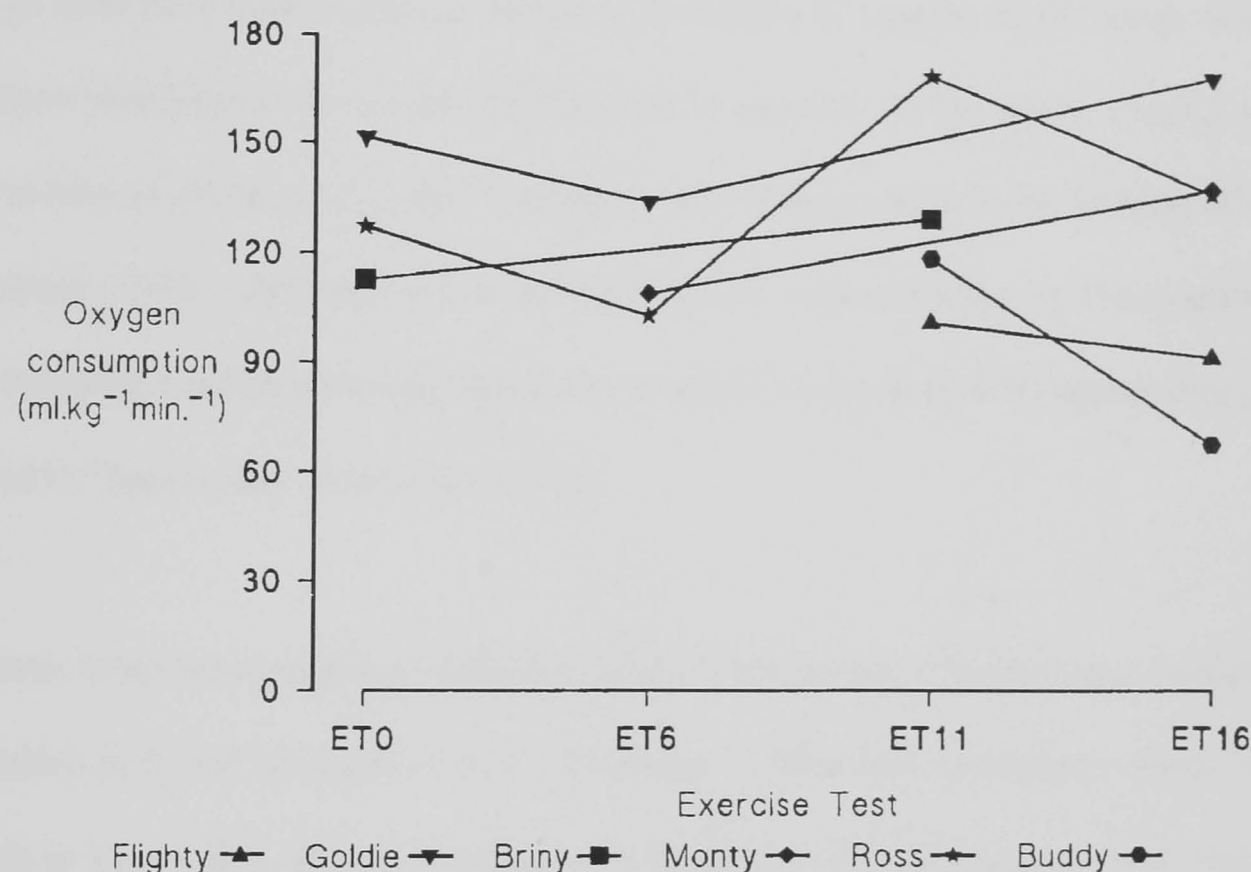
As discussed in Chapter Seven for the fit horses, a number of measurements of  $\dot{V}O_2$  could not be made due to loss of signal or failure of the programme to align the flowmeter and mass spectrometer signals correctly. For this reason, the effect of training on  $\dot{V}O_2$  could not be evaluated satisfactorily. Figure 8.18 presents the individual measurements of  $\dot{V}O_2$  at the end of C12 that were possible for the six horses. Although values from the later tests tended to be slightly higher than in the early ones for four horses, in the other two horses, for whom data were available only for ET11 and ET16,  $\dot{V}O_2$  was lower in the later test. It should, perhaps, be stressed that these measurements do not purport to be of maximal oxygen consumption but merely the  $\dot{V}O_2$  at the end of C12.

In five horses for whom post-C12 blood lactate data were available for both ET0 and ET16, mean blood lactate concentration at this point reduced significantly following training from  $10.5 \pm 2.2 \text{ mmol.l}^{-1}$  to  $7.7 \pm 2.2 \text{ mmol.l}^{-1}$  (in one horse there was insufficient sample for analysis - this horse showed a reduction of 64% in blood lactate between ET0 and ET11). Individual blood lactate reductions varied from 2-74% (median 39%).

## Discussion

Comparison of data between individual studies of the effects of training on physiological function may be confounded by differences in the training techniques and duration as well as the type of animal used and their previous training history. In this study, a training regime similar to that commonly used in Great Britain to train racehorses was used since horses admitted for medical exercise testing in this country are likely to have undergone a similar form of conditioning.

Figure 8.18: Individual values for  $\dot{V}O_2$  during C12 at different stages of training



At the end of the training period, the horses appeared subjectively to be fitter and seemed to handle the exercise test more easily. More objective evidence for their increase in fitness comes from the blood lactate response to exercise which showed a reduction with training. The difficulties of athletic training of horses was evidenced by the loss of two horses to the study due to orthopaedic injuries.

Although there have been numerous studies of the effects of training on the horse, few of these have investigated the ventilatory response to exercise. In this study, training was shown to have no effect on  $V_b$ ,  $f_b$  and  $\dot{V}_e$  during canter. This is similar to the findings of Art and Lekeux (1993) who reported no modification to these variables in Thoroughbred horses following a different training regime (three weeks of treadmill and lunging exercise, followed by three weeks of interval training).

In humans, it has been suggested that training alters the pattern of breathing in favour of a reduction in  $f_b$  and an increase in  $V_t$  (Shephard, 1982). This manoeuvre results in a reduction in  $V_d$  as well as allowing a greater time for alveolar gas exchange on each breath. In the horse, respiration and locomotion are strongly coupled during canter and this removes the option for dramatic change in the relative contribution of  $V_t$  and  $f_b$  to  $\dot{V}_e$  with training. This is reflected by the lack of change in  $V_d/V_t$  with training, findings consistent with those of Pelletier *et al.* (1987) during low-speed studies.

In cantering horses,  $V_t$  is maintained at a smaller proportion of vital capacity than in running humans. A previous report from this laboratory suggested that horses are capable of increasing  $V_t$  during exercise without altering  $f_b$  (Butler *et al.*, 1993b). It was suggested that

they do not do so because the relative energetic cost of breathing (work of breathing [ $W_{\text{m}}$ ] to  $\dot{V}_{\text{O}_2}$  ratio) would be increased by this manoeuvre since  $W_{\text{m}}/\dot{V}_{\text{O}_2}$  has been shown to increase as  $\dot{V}_e$  increases (Art *et al.*, 1990).

It has been suggested (Poon, 1987) that during exercise in man,  $\dot{V}_e$  is set to optimise the balance between the requirements of increased ventilation and minimisation of the work of breathing. There is evidence that similar mechanisms operate in the horse (Wagner, 1988; Bayly *et al.*, 1989). It may be that horses, for whom  $f_b$  is entrained with stride rate during canter - probably in order that locomotion can, to some extent, assist respiration (Bramble and Carrier, 1983; Young *et al.*, 1992) -  $V_t$  does not increase with training because it would not be energetically economical for it to do so.

The effects of training on peak respiratory flow rates do not appear to have been reported previously. Both PIF and PEF were lower following training during the slower canter speeds but were unchanged during C12. This suggests that the measurement of peak flow rates during near maximal exercise is unaffected by state of training.

A greater degree of exercise-induced arterial hypoxaemia occurred during canter following training. Although  $t_{\text{r}}$  was significantly lower at this stage, the change in temperature does not explain the extra fall in  $P_{\text{aO}_2}$  which occurred in the fit animals. As shown in Table 8.1, correcting  $P_{\text{aO}_2}$  for C12 during ET16 to the higher temperatures of ET0 has only a slight effect on  $P_{\text{aO}_2}$  compared to the overall difference between the two tests.

**Table 8.1: The effect of change in blood temperature with training on corrected values of P<sub>a</sub>O<sub>2</sub> and blood pH for C12 in ET16 (end of training).**

	P <sub>a</sub> O <sub>2</sub>	pH <sub>a</sub>	pH <sub>t</sub>
ET16 data corrected for ET16 t <sub>t</sub>	66.7 (±2.8)	7.302 (±.045)	7.189 (±.052)
ET16 data corrected for ET0 t <sub>t</sub>	69.1 (±2.7)	7.295 (±.045)	7.182 (±.053)
ET0 data	81.3 (±5.4)	7.248 (±.044)	7.133 (±.051)

Mean t<sub>t</sub> during C12 was 40.2°C (±0.4) for ET and 39.7°C (±0.2)

The effects of training on exercising P<sub>a</sub>O<sub>2</sub> have been variable in previous studies. Whilst Sexton, Argast and Erickson (1985) found a decrease in P<sub>a</sub>O<sub>2</sub> in exercising ponies following training, Sexton, Erickson and Coffman (1987) reported that training ameliorated the decrease in P<sub>a</sub>O<sub>2</sub> during exercise in Quarter Horses. The finding in ponies is interesting since several studies of untrained ponies have suggested that, unlike horses, they do not demonstrate exercise induced arterial hypoxaemia (Parks and Manohar, 1983; Parks and Manohar, 1984). Thornton *et al.* (1983) found variable changes in P<sub>a</sub>O<sub>2</sub> with training, but no overall trend while Erickson *et al.* (1987) found no effect of training on exercising P<sub>a</sub>O<sub>2</sub>.

The differences between the present findings and those of Thornton *et al.* (1983) may be due to the latters' study being carried out on trotting Standardbreds. The variation in findings between this study and those of Erickson *et al.* (1987) and Sexton, Erickson and Coffman (1987) is unlikely to be due to breed differences since Quarter Horses are very

similar to Thoroughbreds (the Quarter Horse breed being developed from and still frequently crossed with the Thoroughbred). The running speeds in the earlier studies were, however, all considerably lower than in the present one and, although the gait of the animals is not stated it is unlikely that they were cantering at those speeds.

It was not possible to determine why  $P_{aO_2}$  fell with training in this study. The major cause of exercise induced-arterial hypoxaemia in horses has been shown to be alveolar-capillary diffusion limitation with hypoventilation also having an effect (Wagner *et al.*, 1989; Anderson *et al.*, 1989). Since  $P_{aCO_2}$  did not rise with training, there is no evidence that hypoventilation during canter was more marked in the trained animals, whilst  $P_{(a-ET)CO_2}$  difference was also unaltered by training, mitigating against increases in ventilation-perfusion inequalities with training. A widening  $P_{(ET-a)O_2}$  difference during canter with training would suggest an increased diffusion limitation in the trained animals and this variable was significantly higher following training during C12.

One could speculate that trained horses, with a higher  $\dot{Q}_{max}$  might be more prone to develop arterial hypoxaemia during strenuous exercise, when  $\dot{Q}$  approached maximum, since pulmonary capillary transit time would reduce under such conditions. Previous studies have found however, that  $\dot{Q}$  at given sub-maximal work rates was unchanged with training (Bayly, Gabel and Barr, 1983; Thomas *et al.*, 1983). Unfortunately, in this study the effect of training on  $\dot{Q}$  could not be determined since the lack of haemoglobin data for ET0 precluded calculation of  $C_{(a-v)O_2}$  for that test and the patchy nature of the  $\dot{V}O_2$  data allowed derivation of  $\dot{Q}$  using the Fick principle in insufficient cases to draw conclusions for the remaining three tests.

Despite the lower  $P_{aO_2}$  during canter, arterial oxygen saturation was not significantly reduced by training and the increase in haemoglobin concentration with training resulted in an enhanced oxygen carrying capacity per unit volume of blood. This was reflected in the increase in both  $C_{aO_2}$  and  $C_{\bar{v}O_2}$  following training.  $C_{(a-\bar{v})O_2}$  however was unchanged during canter following training. Previous reports have found this latter variable to change upwards, downwards or remain unchanged with training (Bayly, Gabel and Barr, 1983; Thornton *et al.*, 1983; Evans and Rose, 1988b; Knight, Sinha and Rose, 1991).

In this study, blood pH increased with training. Sexton, Argast and Erickson (1985) also found an increase in exercising  $pH_a$  in ponies after training although this finding is not a consistent feature of training studies. The higher *resting* blood pH following training was associated with a slight, but significant decrease in resting blood lactate at that stage (0.5 mmol.l<sup>-1</sup> in ET0 and 0.4 mmol.l<sup>-1</sup> in ET16). The cause of this is uncertain but could be associated with a greater degree of pre-test apprehension in the untrained horses, although an alternative explanation is that there may have been alterations in muscle perfusion brought on by training.

A lower mixed venous temperature during exercise following training was not unexpected, in view of previous findings (Bayly, Gabel and Barr, 1983; Sexton, Argast and Erickson, 1985; Sexton, Erickson, DeBowes and Sigler, 1986; Sexton, Erickson and Coffman, 1987), but the lower temperature at rest is more difficult to explain. ET0 took place in October and ET16 in February when the external temperature was lower, but prior to each resting measurement the horses were standing in the air-conditioned treadmill for approximately one and a half to two hours during preparation for the test.

A brief note is indicated of the changes in respiratory strategy during the warm up trot. Although  $\dot{V}_e$  was not significantly different during trot before and after training,  $V_t$  was significantly higher than in the untrained state.  $f_b$  for this gait was lowest at the end of training, but the decrease was not significant. It is of interest that when moving in a gait in which respiration and locomotion are not entrained, the response to exercise appears to be similar to that reported for man in this respect.  $t_i/t_e$  was significantly greater for trot before training.

In this instance, this change in breathing strategy does not appear to have improved ventilation or gas exchange since  $P_a\text{CO}_2$  (and  $P_{ET}\text{CO}_2$ ) were significantly higher in the post-training tests, while  $P_a\text{O}_2$  and  $S_a\text{O}_2$  were significantly reduced at this stage.

It appears that whilst training results in a significant improvement in  $\dot{V}_{O_{2\max}}$ , this is achieved without a significant increase in pulmonary ventilation during fast exercise. Furthermore, there is no alteration of breathing strategy to decrease  $V_d/V_t$  so as to increase  $\dot{V}_A$  for a given level of  $\dot{V}_e$ . These findings are in agreement with earlier studies which found no increase in  $\dot{V}_e$  (Evans and Rose, 1988b; Art and Lekeux, 1993) or  $V_d/V_t$  (Pelletier *et al.*, 1987) with training. Evans and Rose (1988b) found the increase in  $\dot{V}_{O_{2\max}}$  associated with training to be due to an increase in  $\dot{Q}_{\max}$  produced by an increase in cardiac stroke volume.

In this respect, the horse appears to be similar to man in that training improves cardiovascular and skeletal muscular function with little change in the pulmonary system (Dempsey, 1986). It has been shown in man that highly trained athletes may develop a  $V_t$  of sufficient size to exceed the maximum expiratory flow-volume curve and hence in these



individuals, ventilation becomes limiting to performance (Dempsey, Hanson and Henderson, 1984; Whipp, 1992). Breathing at a lower  $V_t$  may avoid or delay the occurrence of a similar phenomenon in the horse. Further evidence that the pulmonary system is a limiting factor to exercise comes from the finding in both species that breathing helium-oxygen mixtures results in an increase in ventilation compared to air breathing (Dempsey, Hanson and Henderson, 1984; Art and Lekeux, 1989b).

Whipp (1992) suggested that possession of a genetically superior pulmonary system may be a pre-requisite for the elite human athlete to utilise fully the enhancements in cardiovascular and muscular function which result from training. The apparent lack of adaptation of the equine pulmonary system with training suggests that a similar situation is likely in the horse.

With regard to clinical exercise testing of performance horses, this lack of change in pulmonary function is convenient as it circumvents somewhat problems of testing animals at differing stages of fitness. Since horses presented for clinical exercise testing vary in their state of training and subjective assessments of fitness based on visual assessment are likely to be prone to error, it is important to determine the degree to which the results of respiratory function tests are affected by the state of training of the horse.

This study suggests that clinical interpretation of parameters of ventilation such as  $V_e$ ,  $f_b$  and  $\dot{V}_e$  obtained during cantering exercise are uncomplicated by state of training. Similarly, PIF and PEF at the higher levels of exercise ( $12 \text{ m.s}^{-1}$  and greater) appear to be unaffected by training.  $P_{aO_2}$  and blood pH would appear to require a more careful interpretation as these

variables were affected by conditioning, but other blood gas parameters and end-tidal gas tensions were little affected by training.

## CHAPTER NINE - INFLUENZA CHALLENGE STUDIES 1 AND 2 (V1 & V2)

The results of the first two challenge studies are considered together here since they represented the first attempts to perform influenza challenge in Thoroughbred horses having a partial immunity to influenza, conferred by previous but lapsed vaccination. As such, they acted as pilot studies for the third challenge, described in the following chapter.

### Summary of Methods

Three unfit horses were used in each study, two were challenged by aerosol administration of A/equine/2 (H3N8) equine influenza virus<sup>1</sup>, the other was a control animal. Standardised exercise tests (SETs) were performed fourteen days before and twenty one days after influenza challenge. After the pre-challenge SET, the horses were moved to the field station for fourteen days acclimatisation prior to challenge. The horses were moved back to Newmarket on day twenty post-challenge.

SETS were carried out as described in Chapter Three, the speeds used for canter being six, eight and ten m.s<sup>-1</sup> in V1 and six, eight, ten and twelve m.s<sup>-1</sup> in V2.

### Results

#### *Clinical responses to challenge*

Influenza infection was confirmed both by virus isolation and serology in all four challenged horses. Virus was isolated from nasopharyngeal swabs from challenged horses on days 2-3

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<sup>1</sup>For the V1 study the Newmarket/79 strain was used and for V2 the Suffolk/89 strain.

and 2-6 in the first study and on day 2 only in the second study. No virus was isolated from swabs taken from control horses.

HI and SRH antibody titres against influenza are shown in Table 9.1. It will be noted that the control horse in V2 was one of the animals challenged in V1. This was necessary as the intended control horse for V2 became ill just before the study and no other substitute was available. As the two studies were five months apart and the horse showed no indication of respiratory disease during the interim period, it was felt that he was admissible as a substitute control animal.

All challenged horses, but no control animals, showed significant increases in serum antibody activity against the A/equine/2 test strains, whilst one, Monty, also showed an increase in A/equine/1 antibody (a cross reaction of this type between strains is not infrequently seen in previously-vaccinated horses).

Clinical signs of infection were mild. A transient pyrexia response (rectal temperature not less than 38.9°C) occurred in three challenged horses on day 2 post-challenge<sup>2</sup> only. The fourth horse (Spock in V2) did not demonstrate a significant pyrexia, his highest rectal temperature being 38.7°C on day 7. All challenged horses showed mild, infrequent coughing of two to nine days duration and a slight nasal discharge on occasional days during the first week.

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<sup>2</sup>Mean rectal temperature on day 2 was 38.8°C (range 37.8-39.3°C).

**Table 9.1: Pre- and post-challenge influenza serum SRH and HI antibody titres for the horses in studies V1 and V2**

		HI titres			SRH levels		
		E1/P	E2/M	E2/F	E1/P	E2/M	E2/F
V1							
Hero	Day 0	128	256	128	125.7	58.0	79.5
	Day 14	128	<b>1024</b>	<b>1024</b>	125.7	<b>136.1</b>	<b>167.2</b>
Merry Ridge	Day 0	64	256	32	106.0	44.5	51.0
	Day 14	128	<b>1024</b>	<b>1024</b>	79.5	<b>123.6</b>	<b>149.0</b>
Killer*	Day 0	256	512	256	160.3	82.9	96.8
	Day 14	256	1024	256	158.0	69.9	86.2
V2							
Monty	Day 0	N/D	16	8	6.8	11.8	N/D
	Day 14	N/D	<b>1024</b>	<b>1024</b>	<b>56.6</b>	<b>125.7</b>	<b>181.5</b>
Spock	Day 0	64	512	256	123.6	53.8	100.4
	Day 14	16	1024	<b>1024</b>	131.9	<b>121.6</b>	<b>191.4</b>
Merry Ridge*	Day 0	64	1024	1024	96.8	113.6	153.5
	Day 14	64	1024	1024	106.0	129.8	158.0

\* = control horse

Figures in bold indicate a significant increase in serum antibody titre

N/D = no antibody detectable

E1/P = A/equine/1/Prague/56-E2/M = A/equine/2/Miami/63-E2/F = A/equine/2/Fontainebleau/79

One horse (Spock in V2), continued to show a very slight nasal discharge until day 18, but this may have been due to a mild traumatic rhinitis as a result of the nasopharyngeal swabbing as the V2 control horse also showed a similar discharge on days 5 and 10-17 and such a finding has been noted in previous challenges (Hannant, D., personal communication). All of the horses continued to eat throughout the study and none showed more than very mild depression during the first week.

Haematological changes were also slight. Total leucocyte counts were not elevated at any stage. In V1, Hero showed a neutropaenia on days one and two, followed by a slight to moderate lymphopaenia between days four to nine and twelve to thirteen, whilst only a slight lymphopaenia occurred in Merry Ridge on days five and seven. In V2 none of the changes were marked, but Monty showed an eosinophilia on day two, lymphopaenia on days two and three and neutropaenia on days five, six, eleven and thirteen. In Spock, a neutropaenia occurred on day one, a leucopaenia on day two, a lymphopaenia on day three and neutropaenia on days ten, eleven and thirteen. Slight increases in plasma viscosity (PV) (1.63-1.70 cP) occurred in all four horses<sup>3</sup>.

TW and BAL both revealed the presence of severe, acute airway inflammation on days three and seven in challenged horses, as evidenced by raised nucleated cell counts and increased proportions of neutrophils.

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<sup>3</sup>PV was elevated on the following days:

Hero	- days five, seven and eight
Merry Ridge	- days eight and nine
Kirk	- days five to nine; twelve to fourteen
Spock	- days eight and eleven

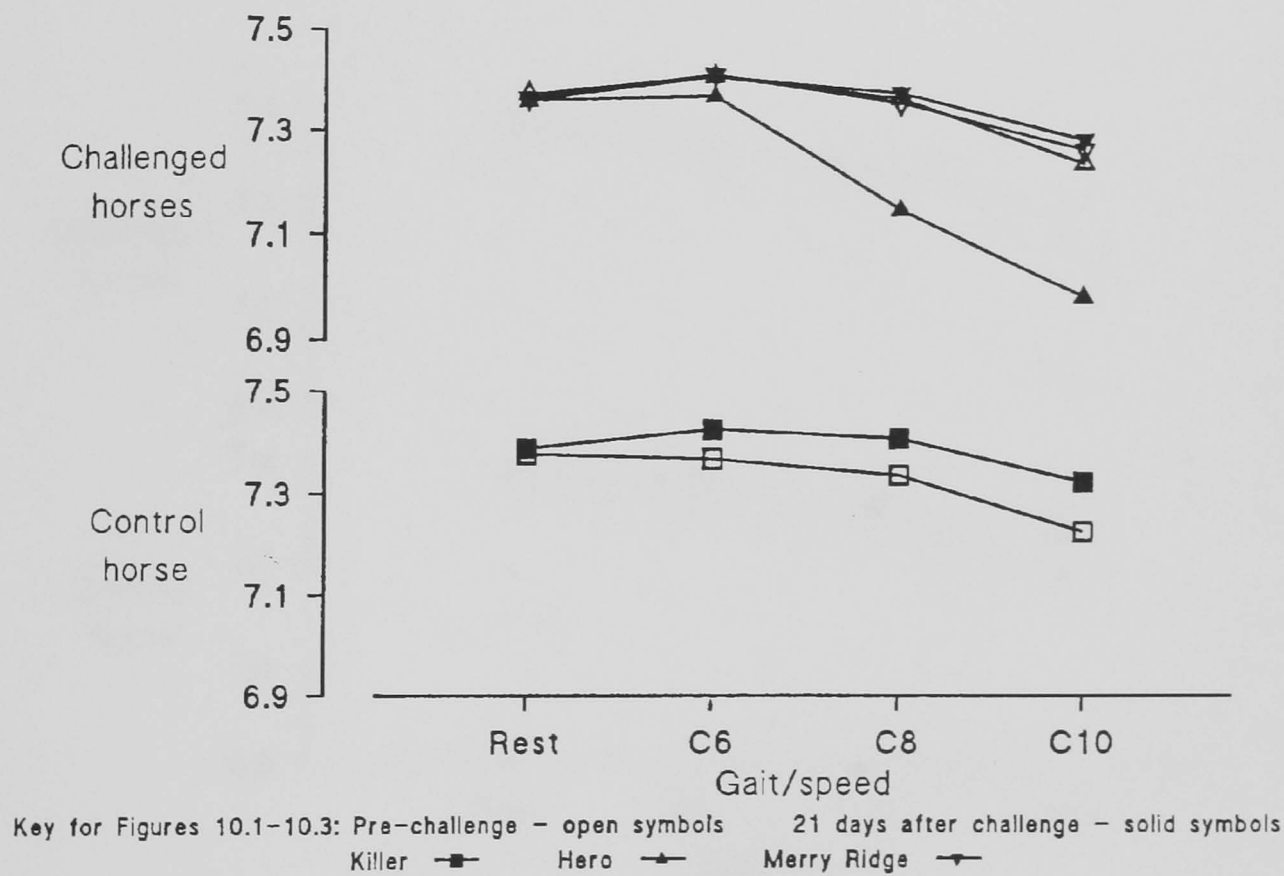
### *Exercise testing*

All horses were able to complete the post-challenge SETs without any apparent discomfort.

The respiratory response to exercise was qualitatively similar to that seen in the studies on normal horses (Chapter Seven). In the challenged horses, no definite trends towards an altered response following influenza infection could be discerned with regard to  $\dot{V}_e$ ,  $f_b$ ,  $V_t$ , PIF, PEF,  $P_{ET}O_2$ ,  $P_{ET}CO_2$ ,  $\dot{Q}$ ,  $\dot{V}O_2$ ,  $C_aO_2$ ,  $C_{\bar{v}}O_2$ ,  $C_{(a-\bar{v})}O_2$  or  $t_{\bar{v}}$  (either with respect to their own pre-challenge tests or to the data from the control horses). This remained the case whether the two challenges were considered separately or together. For all of these variables, a paired, two-tailed Student's *t* test for pooled data from the four challenged animals showed no significant differences between pre- and post-challenge tests.

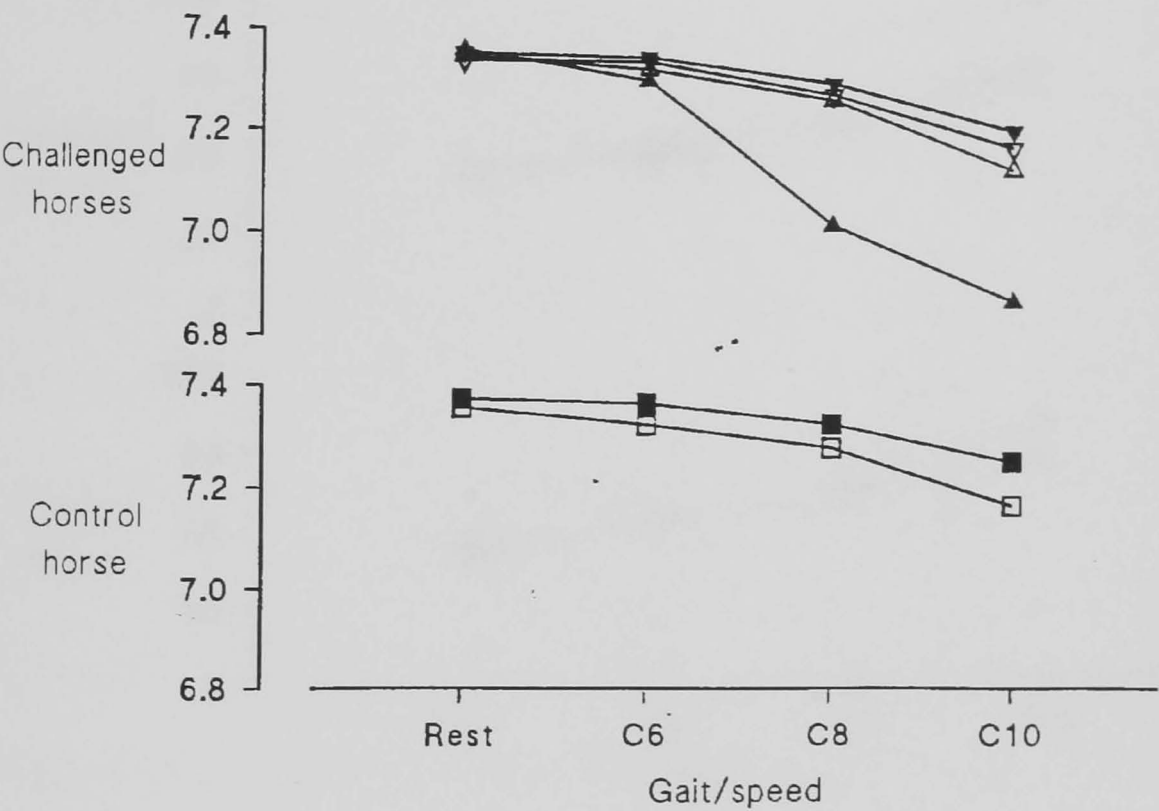
Due to the blood gas analyser malfunctioning on the morning of the Day 21 test for V2, blood gas data were available only for the first challenge. In this study, one of the challenged horses, Hero, showed a markedly lower blood pH during C8 and C10 (the highest workloads in this study) in the Day 21 test, both for arterial and mixed venous blood (Figures 9.1 and 9.2). At 10 m.s<sup>-1</sup>, pH<sub>a</sub> and pH <sub>$\bar{v}$</sub>  were respectively 7.243 and 7.125 prior to challenge and 6.987 and 6.869 on Day 21. This was associated with an increase in P <sub>$\bar{v}$</sub> CO<sub>2</sub> on Day 21 with respect to pre-challenge results (Figure 9.3). This horse also showed a marked increase in blood lactate concentration following exercise in the Day 21 SET compared to the pre-challenge test (blood lactate concentration was 10.7 mmol.l<sup>-1</sup> at 10 m.s<sup>-1</sup> before challenge and 25.5 mmol.l<sup>-1</sup> on Day 21).

**Figure 9.1: Changes in arterial pH during exercise before and 21 days after influenza challenge**

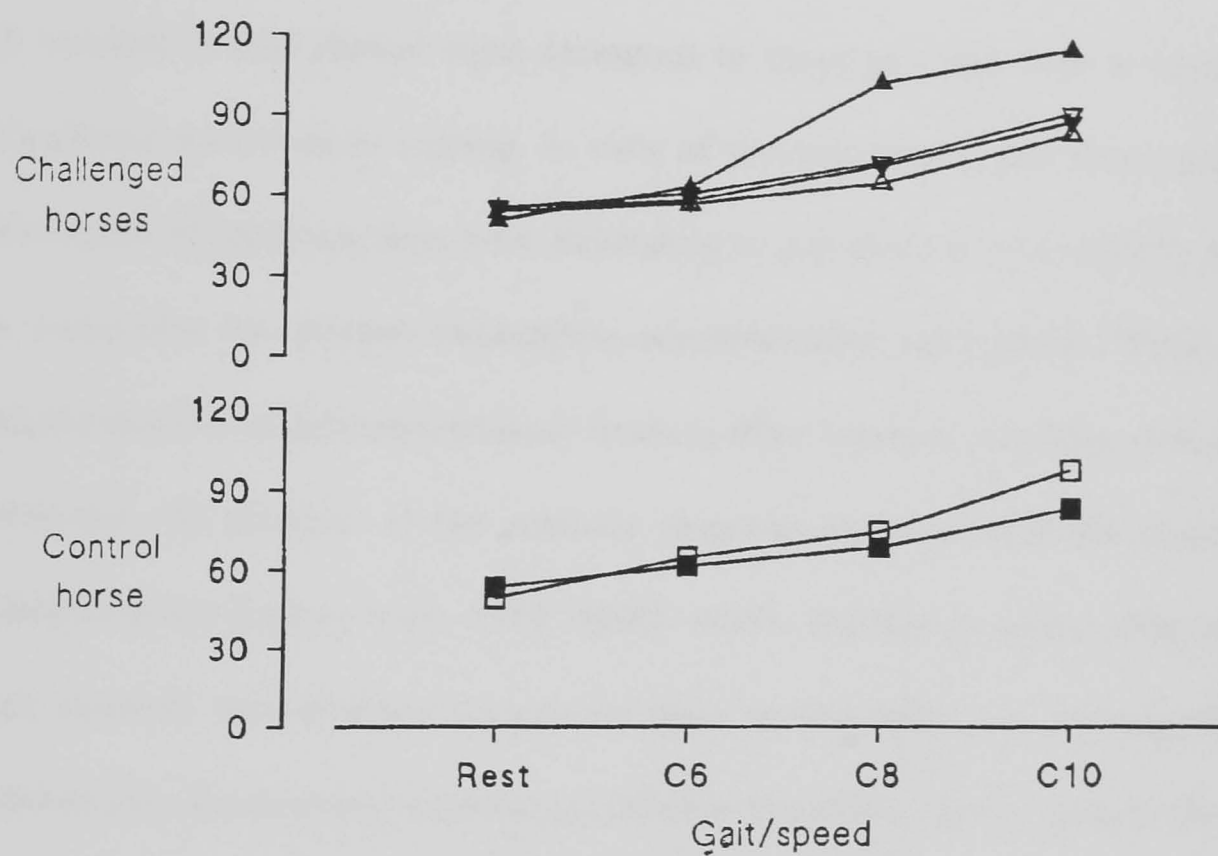




**Figure 9.2: Changes in mixed venous pH during exercise before and 21 days after influenza challenge**



**Figure 9.3: Changes in  $P_{\bar{v}CO_2}$  (mm Hg) during exercise before and 21 days after influenza challenge**



## Discussion

Discussion of the results of these studies will for the most part be limited here to consideration of factors affecting the design of the third challenge study, the results of which are presented in Chapter Ten. A discussion of the implications of all three challenges will follow in that Chapter.

In these partially immune horses it was possible to induce influenza infection at a level which resulted in mild clinical signs analogous to those generally seen in vaccinated Thoroughbred racehorses in training. In view of previous reports that serum antibody levels to equine influenza are short-lived, diminishing to unprotective levels as early as four to six weeks after two primary vaccinations administered to naive ponies (Wood *et al.*, 1983b), the duration of influenza antibody levels in these horses is surprising. It has been reported that the duration of the antibody response increases with the number of vaccinations given (Ingram *et al.*, 1978; WHO, 1983). The horses used in these studies had all received several doses of vaccine prior to the period of non-vaccination, nevertheless they demonstrated a greater persistence of antibody than expected, although all four of the challenged horses had initial serum antibody titres well below the levels reported to be necessary to protect against aerosol challenge<sup>4</sup> (Table 9.1).

The respiratory variables measured in these studies were found to be unaffected three weeks after infection with influenza resulting in low-grade disease similar to that occurring naturally in vaccinated, racing Thoroughbreds. It has often been suggested that horses

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<sup>4</sup>SRH titres of 120-154mm<sup>2</sup> (Mumford, 1992).

recover more quickly and more fully from respiratory viral infections when allowed complete rest throughout the course of the disease. These initial results support this claim, however they fail to take into account the effect of physical training up to the time of challenge (see below), which would, of course, occur with infections in horses in training.

The only notable alteration in the measured parameters was the marked reduction in blood pH after challenge in one animal. This appears likely to be related to a markedly greater blood lactate response to exercise in this test. As respiration was unaltered in this horse following challenge, it seems likely that these changes resulted from peripheral factors such as alterations in muscle perfusion or metabolism.

Hero showed no evidence of lameness or muscular stiffness following the 21 day test. His results are however, of interest since it is thought that the incidence of equine rhabdomyolysis syndrome (ERS) may increase in stables where recent outbreaks of viral respiratory disease have occurred (Gerber, 1970). Although for the most part evidence for this is anecdotal, Harris (1990) reported such an occurrence following an outbreak, in a Newmarket racing stable, of respiratory disease due to Equine Herpesvirus 1.

Unfit horses were used for these studies so that the period of enforced confinement to their loose boxes at the field station would not produce a detraining effect which might in itself alter respiratory function. The canter speeds used in V1 ( $6-10 \text{ m.s}^{-1}$ ) were selected as representing work loads which it would be possible for unfit animals to perform without excessive risk of musculoskeletal injury due to lack of conditioning. As the horses in the V1 study performed these speeds so easily, an extra canter speed was added for V2 (12

m.s<sup>-1</sup>) to produce a greater demand on the respiratory system in an attempt to increase the chance of detecting subtle alterations in respiratory function.

Since both of these studies revealed little alteration in the variables measured following influenza infection, it was decided that for further challenges more radical modification in the protocol was indicated. Since it is generally believed that horses in training are more susceptible to respiratory infections, it was decided that the next study should be performed using horses in full training up until the time of viral challenge.

As there were no facilities at the containment unit for exercising the infected animals, they would have to rest during the period of infection, however a period of fifteen weeks out of training did not significantly alter respiratory function in an earlier study carried out in this laboratory (Butler *et al.*, 1991). In any case, rest during the acute period of disease would model the situation in horses in training who should be rested with the onset of disease. In order to assess the effects of the three week period of inactivity at the field station a control group of horses was used to assess the affect of this on the variables measured in the exercise tests.

A brief discussion of the effects of training on the incidence and severity of respiratory disorders is of value at this stage.

#### *The influence of training on the occurrence and severity of respiratory disease*

There is some controversy as to the effect of athletic training on immune function in man (Cannon, 1993). Jackson, Dowling, Anderson, Riff, Saporta and Turck (1960) found 'an

insignificant increase' in the incidence of colds in subjects exercised following challenge with filtered nasal secretions from naturally-occurring cold cases. In twelve year old children and young adult men, no association was found between the incidence of respiratory infections and the level of sports activity (Osterback and Qvarnberg, 1987; Schouten, Verschuur and Kemper, 1988). In the latter of these studies, a weak but significant relationship was found in women between a higher level of physical activity and a reduced incidence of infection, whilst mildly obese, sedentary women undergoing gentle exercise, reported a reduction in duration of symptoms (but not of number of infections) compared to a sedentary control group (Nieman, Nehlsen-Cannarella, Donohue, Chritton, Haddock, Stoute and Lee, 1991).

It appears that the amount and intensity of exercise is an important determinant of the effect on immunity, thus a single bout of strenuous exercise may suppress immune function transiently and intense exercise may cause a long term immunosuppression, whilst moderate conditioning may enhance immune function (Fitzgerald 1988).

Although marathon runners reported an increased incidence of respiratory infections around the time of events (Peters and Bateman, 1983; Nieman, Johanssen, Lee and Arabatzis, 1990), Cannon (1993) drew attention to problems in the design of these studies. He also noted their similarity to work which has shown a relationship between psychological stress factors and the incidence of disease (Rabkin and Struening, 1976) and of viral shedding in experimental rhinovirus challenge (Totman, Kiff, Reed and Craig, 1980).

It is generally believed that horses in training have an increased susceptibility to respiratory infections (Keadle, Pourciau, Melrose, Kammerling and Horohov, 1993). Huston, Bayly, Liggitt and Magnuson (1987) suggested that the high incidence of respiratory disease in such animals resulted from 'stress', exposure to influenza virus and invasion of bacterial opportunists. Although this view somewhat simplifies the situation, for instance there are other viral agents in addition to influenza capable of causing equine respiratory disease, many clinicians agree that strenuous training is attended by an increased susceptibility to respiratory disease.

In the 1989 influenza outbreak in this country, the incidence of infection (defined as viral replication, detected by virus isolation) and the incidence of disease (the occurrence of clinical signs) were both considerably higher in a stable where horses were kept in work following booster vaccinations than in another yard where horses were vaccinated and rested (Wood, J., personal communication).

Numerous equine studies have shown that exercise results in an increase in circulating cortisol (Church, Evans, Lewis and Rose, 1987; Huston *et al.*, 1987; Keadle *et al.*, 1993) and BAL fluid cortisol (Huston *et al.*, 1987), however resting plasma cortisol was found to decrease with race training (Wilson, Kingery and Snow, 1991). With regard to more specific tests of immune function, Huston *et al.* (1987) reported impaired alveolar macrophage function following a single strenuous bout of exercise in Thoroughbreds. Phagocyte viability remained depressed five days after exercise. Keadle *et al.* (1993) found that a single strenuous exercise test resulted in enhanced lymphokine activated killer cell activity but impaired antigen-specific and non-specific lymphoproliferation (using influenza

A/equine/2 as the specific antigen).

In summary, although the true nature of the relationship between exercise, training and infection is as yet unclear, there is some evidence of exercise-induced alterations in equine immune function and horses in training do appear to be more susceptible to respiratory disease than sedentary individuals. It must however be borne in mind that the horse in active training will have more contact with other animals than the sedentary horse and will therefore be exposed to a greater risk of encountering pathogens.

\* \* \*

An additional advantage accruing from the use of fully-trained horses for the V3 study was that it permitted a still more strenuous exercise test to be used than in V1 and V2.

The design of the challenge studies was influenced by the housing facilities available at the containment unit where there were only three loose boxes suitable for Thoroughbred horses. For this reason each of the first two studies comprised two subjects and one control horse and the control animal was tended and examined each day prior to contacting the challenged animals.

For the third study, the fully-conditioned horses from the training study were used. It was necessary that the study be completed in as short a time as possible due to availability of facilities and since keeping the group in training for a prolonged period would increase the risk of loss of animals due to injury. Additionally, completing the study as quickly as



possible minimises variations in fitness during the study.

Hence, the third challenge study involved two groups of three horses coming out of the training study in full work. Initially one group was taken to the field station and challenged whilst the other acted as a control group to assess the effects of the period of inactivity which was unavoidable for the challenged horses. The control group were then put back into work for a further two weeks before themselves undergoing an influenza challenge. This study is described in the next chapter.

## CHAPTER TEN - INFLUENZA CHALLENGE STUDY 3 (V3)

The third of the challenge studies is described here. For reasons described in the previous chapter, in this study horses were used which were fully trained up until the time of challenge.

### Summary of Methods

The six horses which completed the training study and were considered to be in full training were used for this study. They were divided into two groups of three. One group (Group 1) was removed to the field station and subjected to challenge with influenza (the A/equi/2/Suffolk/89 strain was used on this occasion, since the vaccines previously used on these horses did not contain this particular strain of virus) as in V1 and V2 except that in the present study, the horses were left in the challenge box for two hours rather than one to prolong exposure to aerosolised virus. The other three horses (Group 2) were moved to a separate site and kept as a control group under similar conditions to the challenged horses. Exercise tests were performed on the challenged horses on Day 21 and on the control horses the following day.

The Group 2 animals were then put back into work for another two weeks before being taken to the field station for influenza challenge using the same form of challenge as Group 1. Due to a shortage of available horses, it was necessary to use a horse (Monty) in this study who had been used in the V2 challenge study and had therefore, been infected with influenza the previous year. This horse was in Group 2. The design of the study is shown in Table 10.1.

**Table 10.1: Study design for V3 influenza challenge study**

Day:	-1&-2	0	21-22	35-36	57
Group 1	↑	▲	↑		
Group 2		*	*	↑ ▲	↑

▲ = influenza challenge

↑ = exercise test on challenged group

\* = exercise test on control group

**Results**

*Clinical responses to challenge*

*(i) Control Group<sup>1</sup>*

Although during their period as control animals the Group 2 horses showed no rise in serum antibodies to influenza (Table 10.2) and no virus was isolated from nasopharyngeal swabs, one horse (Buddy) showed a persistent, mild, mucoid nasal discharge and tracheal wash cytology showed evidence of airway inflammation in the absence of significant bacterial pathogens. He showed no other clinical signs of disease and it appears likely that this horse was suffering from low-grade chronic obstructive pulmonary disease (COPD) and that his signs were precipitated by the change of environment and exposure to allergens to which he was hypersensitive.

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<sup>1</sup>Group 2 horses during the control period.

**Table 10.2: Pre- and post-challenge influenza serum SRH and HI antibody titres for the horses in V3**

	HI titres			SRH titres		
	E1/P	E2/M	E2/F	E1/P	E2/M	E2/F
<i>First challenge group</i>						
Flighty						
Day 0	64	128	64	113.6	63.8	N/D
Day 14	8	32	16	98.67	40.7	N/D
Goldie						
Day 0	8	64	8	100.4	37.1	N/D
Day 14	<b>32</b>	<b>1024</b>	<b>1024</b>	<b>167.2</b>	<b>167.2</b>	<b>160.3</b>
Briny						
Day 0	64	256	128	179.1	77.8	115.6
Day 14	128	<b>1024</b>	<b>1024</b>	204.1	<b>158.0</b>	<b>181.5</b>
<i>Second Challenge Group</i>						
Monty						
Day 0	N/D	32	16	11.0	39.5	N/D
Day 14	N/D	32	16	14.2	39.5	N/D
Buddy						
Day 0	32	1024	256	162.6	109.8	115.6
Day 14	64	1024	128	171.9	117.6	109.8
Ross						
Day 0	16	512	64	68.3	94.9	106.0
Day 14	16	512	64	81.1	102.2	<b>155.7</b>
<i>Control Group</i>						
Monty						
Day 0	N/D	32	16	11.0	39.5	N/D
Day 14	N/D	32	16	11.0	39.5	N/D
Buddy						
Day 0	32	1024	256	162.6	109.8	115.6
Day 14	64	1024	128	162.6	109.8	115.6
Ross						
Day 0	16	512	64	68.3	94.9	106.0
Day 14	16	512	64	68.3	94.9	106.0

Figures in **bold** indicate a significant increase in serum antibody titre

N/D = no antibody detectable

E1/P = A/equi/1/Prague/56 - E2/M = A/equi/2/Miami/63 - E2/F = A/equi/2/Fontainebleau/79

### *(ii) Challenged Horses<sup>2</sup>*

Virus was isolated from nasopharyngeal swabs in only four challenged horses, all three animals in Group 1 and one in Group 2. In three horses, virus was isolated only on day two, in the other on days two and five. Serum HI and SRH antibody titres are shown in Table 10.2, a significant rise in serum antibody titres occurred in three of the five horses being challenged for the first time. Monty (the horse undergoing a second challenge) did not show a rise in antibody titres.

As in the earlier challenges, clinical signs of infection were for the most part mild, with low-grade coughing and slight mucoid nasal discharge for the first few days in the horses undergoing first challenge. A transient pyrexia was present in the Group 1 horses around day 2, but rectal temperatures remained normal in the Group 2 horses.

One horse (Goldie) in Group 1, was notably lethargic and slow to recover following endoscopy on day seven. She was reluctant to eat on this day. In view of these clinical signs and the second fever spike occurring on this day, it was considered likely that she was suffering from a secondary bacterial infection and that antimicrobial therapy was indicated. This consisted of intravenous sulphadoxine and trimethoprim twice daily for five days (*Borgal 24% Solution* containing 200 mg/ml sulphadoxine and 40 mg/ml trimethoprim, given at a dose rate of 25 mg active ingredients per kilogram bodyweight; Hoechst Animal Health, Milton Keynes). On day eight, she was considerably brighter and she recovered clinically over the next two or three days.

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<sup>2</sup>Group 1 horses plus Group 2 horses during their challenge period.

### *Exercise Testing*

The control horses completed the entire test before and after the control period. Four of the five 'first challenge' horses completed the post-challenge test, but one, Ross was unable to complete more than 1 minute 21 seconds of the final, C12+3 canter. Monty (the horse undergoing a second challenge) did not carry out the final canter (C12+3) as he was felt not to be fit to continue. In retrospect, the decision to stop Monty early may have been erroneous and overcautious as he recovered very quickly after being taken off the treadmill!

The regularity of the pattern of breathing during canter was assessed by determining the proportion of normal breaths (in terms of flow pattern, as defined in Chapter Seven) during each canter and checking for maintenance of 1:1 respiratory:locomotory synchrony. The proportion of normal breaths in each canter is shown in Table 10.3.

The pattern of breathing in the control horses showed little change between the pre- and post-control tests, 1:1 respiratory:locomotory synchrony being maintained and the majority of breaths following the 'normal' pattern.

In the pre-challenge tests, five of the six horses showed a normal pattern of breathing during canter, with rarely less than 90% of normal breaths in any canter. The exception was Buddy, who showed a relative decrease in proportion of normal breaths during C8, C10 and C12 (76.2%, 75.9% and 80.2% respectively) in his pre-challenge test. Following challenge, Buddy's proportion of normal breaths during canter had returned to more usual values.

**Table 10.3: Proportion (%) of normal breaths during canter in the V3 challenge tests**

<i>First Challenge Group</i>		C8	C10	C12	C12+3°
Flighty	Day 0	99.6	99.2	98.0	97.0
	Day 21	<b>47.7</b>	90.0	97.2	98.0
Goldie	Day 0	98.8	99.6	98.7	99.6
	Day 21	98.6	97.8	97.4	98.4
Briny	Day 0	94.1	92.8	89.7	96.3
	Day 21	93.3	98.3	97.5	97.5
<i>Second Challenge Group</i>					
Monty	Day 0	93.4	98.6	97.7	99.2
	Day 21	94.2	97.3	99.1	
Buddy	Day 0	76.2	75.9	80.2	96.5
	Day 21	<i>92.0</i>	<i>98.8</i>	<i>95.7</i>	96.5
Ross	Day 0	94.6	97.4	98.3	93.2
	Day 21	<b>83.6</b>	<b>79.1</b>	92.7	95.3
<i>Control Group</i>					
Monty	Day 0	89.8	99.1	97.9	100.0
	Day 21	93.7	98.2	99.2	98.3
Buddy	Day 0	94.8	91.5	95.9	96.9
	Day 21	100.0	99.2	96.8	97.3
Ross	Day 0	85.0	91.4	97.4	93.4
	Day 21	97.0	98.2	97.8	96.5

Numbers in **bold** indicate value 10% or more below corresponding Day 0 value.

Numbers in *italic* indicate value 10% or more above corresponding Day 0 value.

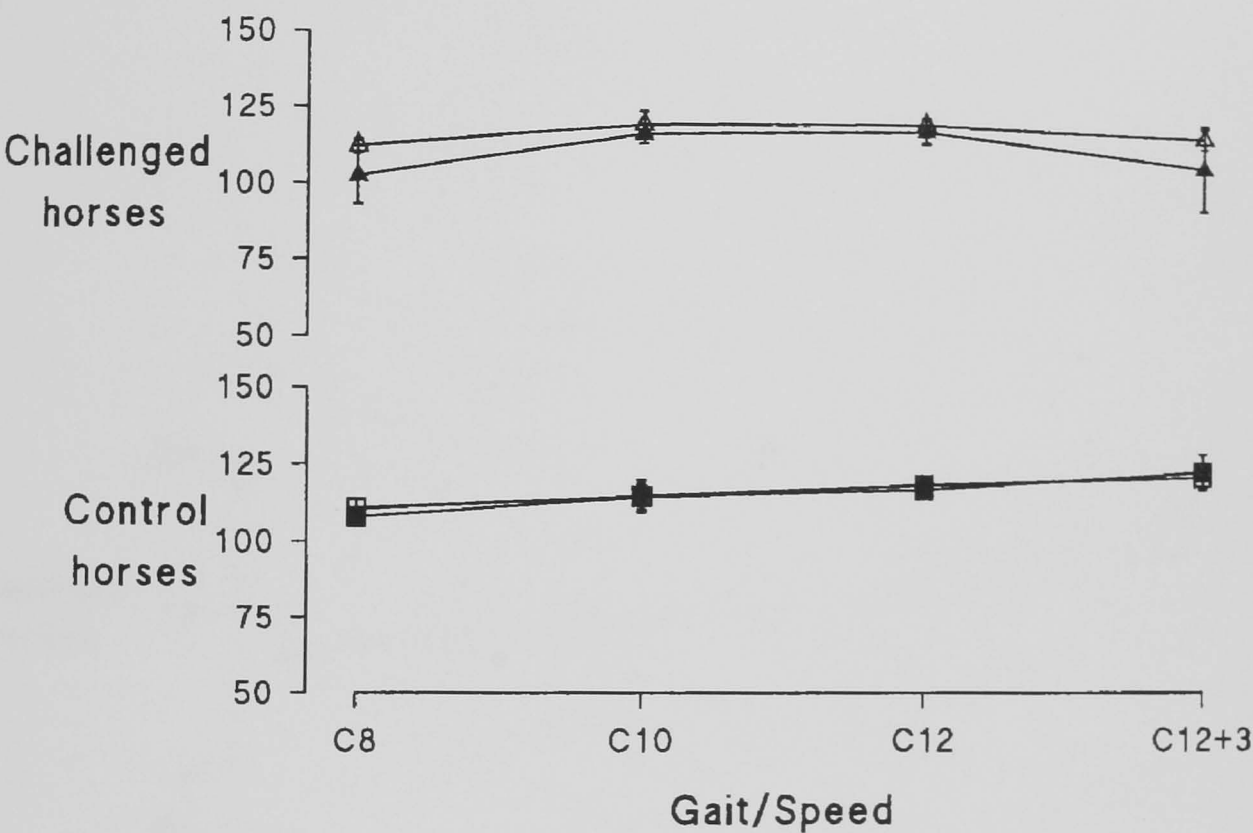
Of the five horses who showed a normal pattern of breathing in their pre-challenge tests, three maintained this regularity following challenge. A reduction in the proportion of normal breaths was seen in C8 and C10 for Ross (83.6% and 79.1% respectively) and a dramatic reduction occurred in C8 (47.7%) for Flighty. During the latter's C8 and C12+3 canters the ratio of  $f_b$  to stride frequency was 1:2, hence  $f_b$  was reduced and  $V_t$  increased.

Other than these slight changes in the pattern of breathing, the respiratory response to exercise following influenza challenge was, again, qualitatively similar to that seen in the studies of normal horses. For the challenged horses, a paired, two-tailed Student's  $t$  test showed no significant differences between pre- and post-challenge tests for  $V_t$ ,  $f_b$ ,  $\dot{V}_e$ , PIF, PEF,  $P_{ET}O_2$ ,  $P_{ET}CO_2$ ,  $\dot{Q}$ ,  $\dot{V}O_2$ ,  $C_aO_2$ ,  $C_{\bar{v}}O_2$ ,  $C_{(a-\bar{v})}O_2$ ,  $P_aO_2$ ,  $P_{\bar{v}}O_2$ ,  $P_aCO_2$ ,  $P_{\bar{v}}CO_2$ ,  $pH_a$ ,  $pH_{\bar{v}}$  or blood lactate concentration during any of the canters. This was the case whether all challenged horses were included or if the horse undergoing second challenge (Monty) or the horse showing abnormalities of pattern of breathing on the pre-challenge test (Buddy) were excluded. Examples of these variables are shown in Figures 10.1-10.9.

In Figure 10.1 the slight (insignificant) reduction in post-challenge mean  $f_b$  notable for C8 and C12+3 occurs due to the influence of the reduced  $f_b$  in Flighty associated with the change in respiratory:locomotory rate ratio.

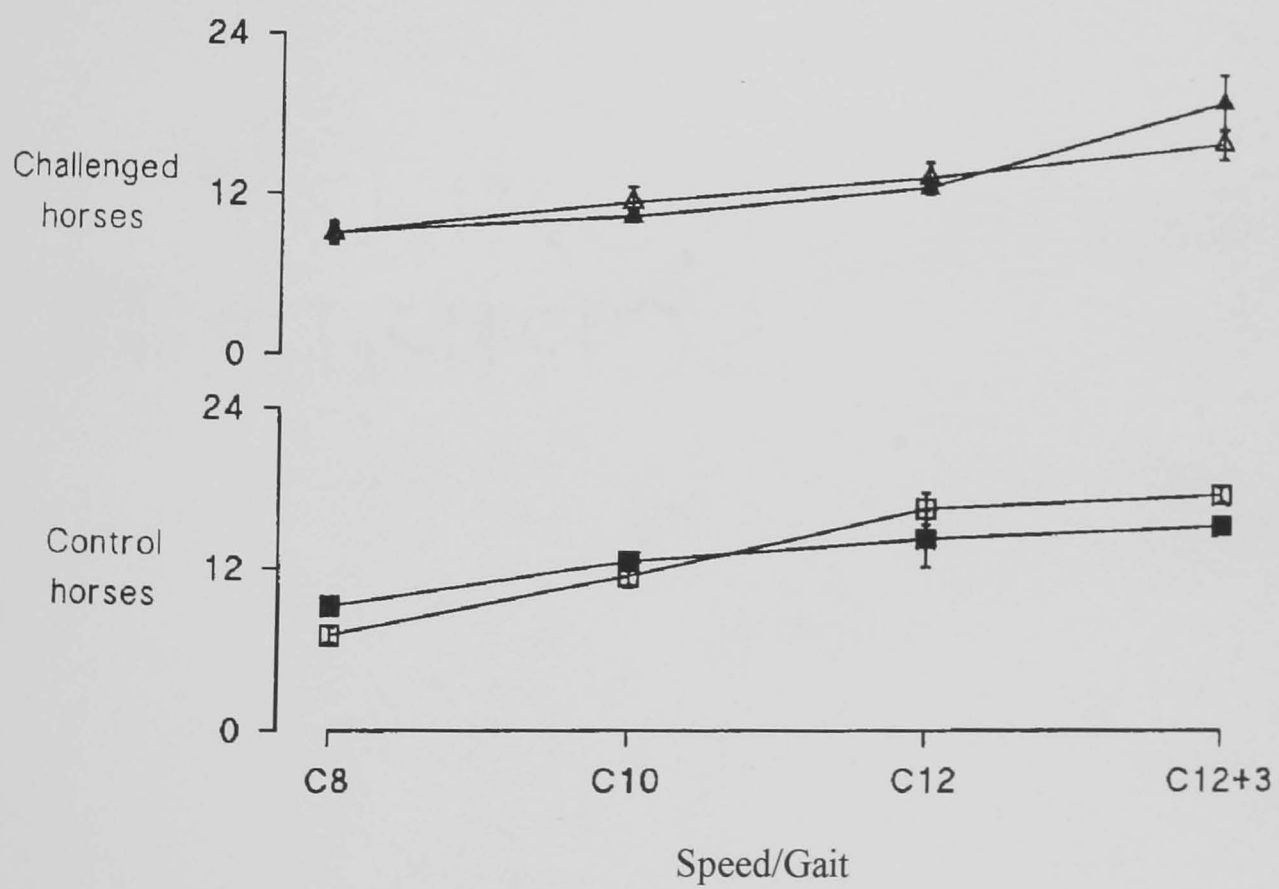


**Figure 10.1: Respiratory frequency (breaths.min<sup>-1</sup>) during exercise in Thoroughbred horses before and 21 days after influenza challenge**

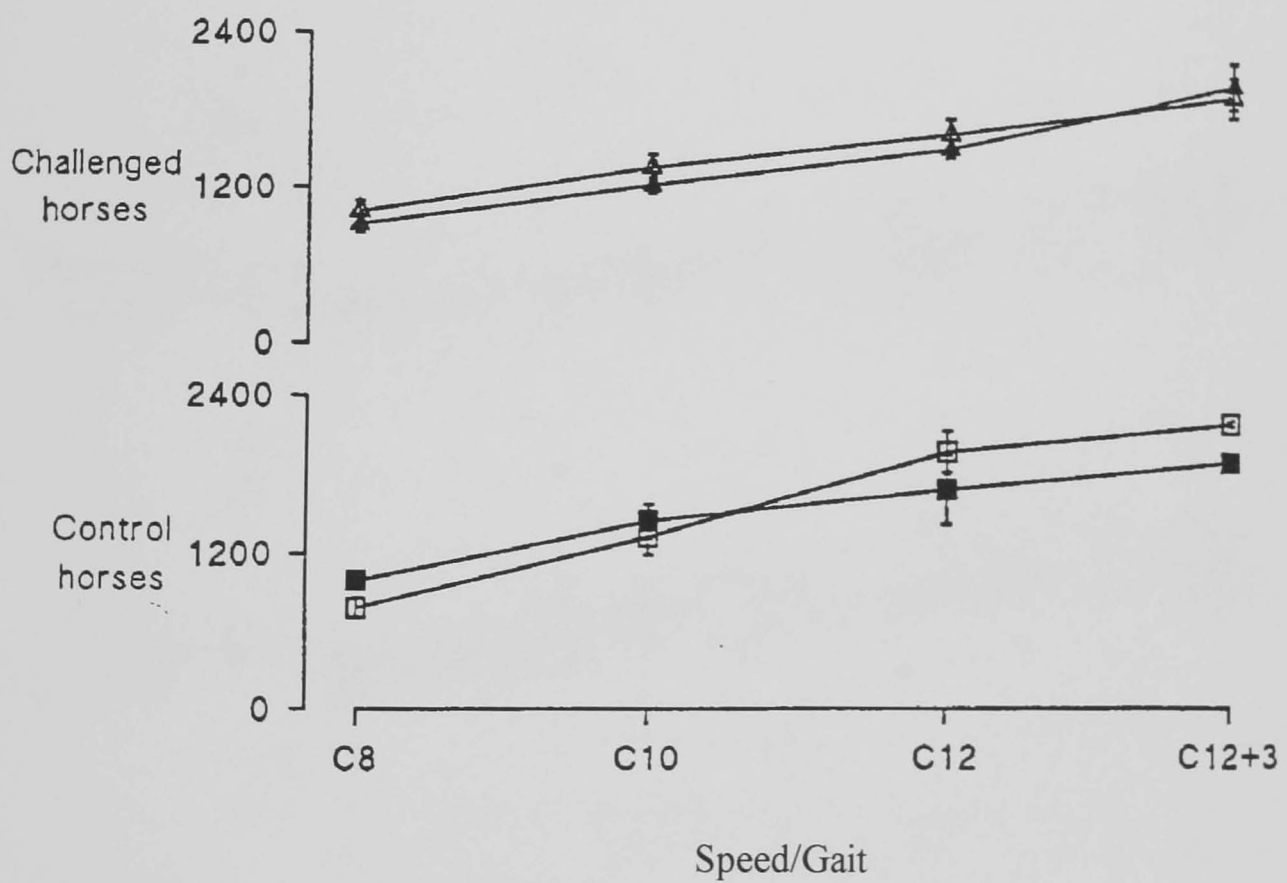


*Figures 10.1 to 10.9 show mean values ( $\pm$ SEM) for variables measured at the end of the canters. Open symbols refer to pre-challenge or pre-control period values and filled symbols to Day 21 post-challenge or Day 22 of the control period. The control horses are represented by square symbols and the challenged horses by triangles.*

**Figure 10.2 Tidal volume (l) during exercise in Thoroughbred horses before and 21 days after influenza challenge**



**Figure 10.3: Minute volume ( $1.\text{min}^{-1}$ ) during exercise in Thoroughbred horses before and 21 days after influenza challenge**



**Figure 10.4: Peak respiratory flow rates ( $l.s^{-1}$ ) during exercise in Thoroughbred horses before and 21 days after influenza challenge**

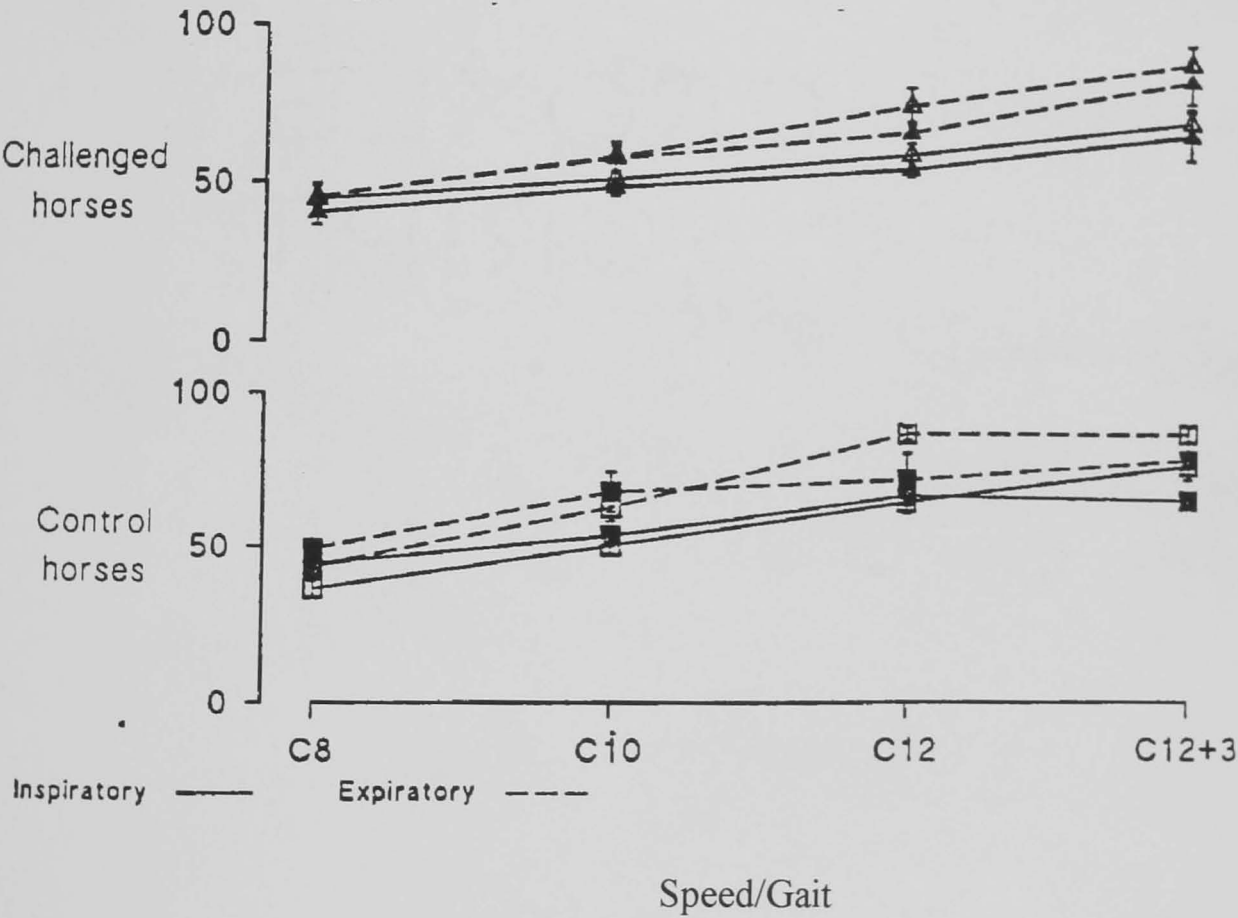


Figure 10.5:  $P_{O_2}$  (mm Hg) during exercise in Thoroughbred horses before and 21 days after influenza challenge

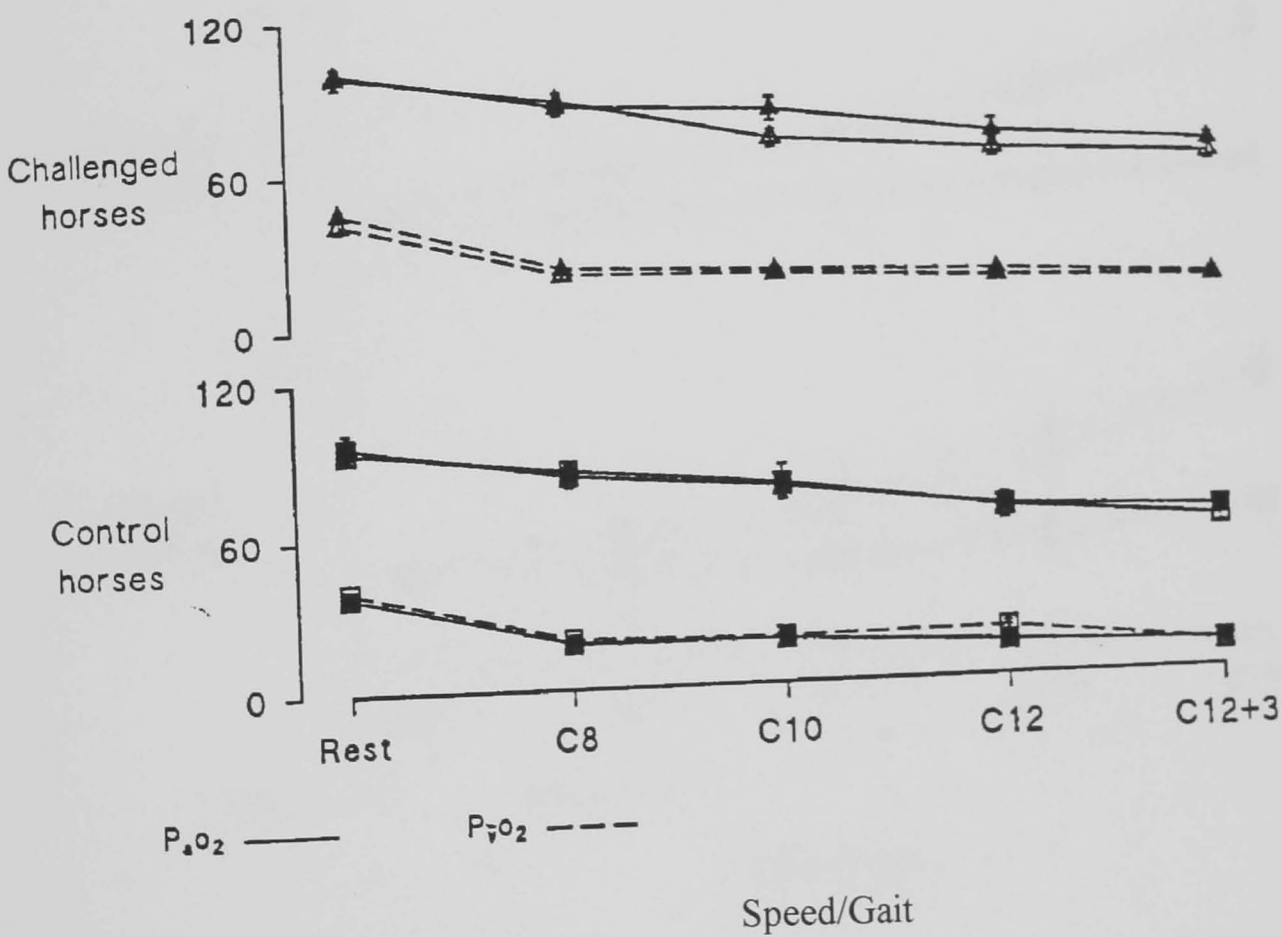


Figure 10.6:  $P_{CO_2}$  (mm Hg) during exercise in Thoroughbred horses before and 21 days after influenza challenge

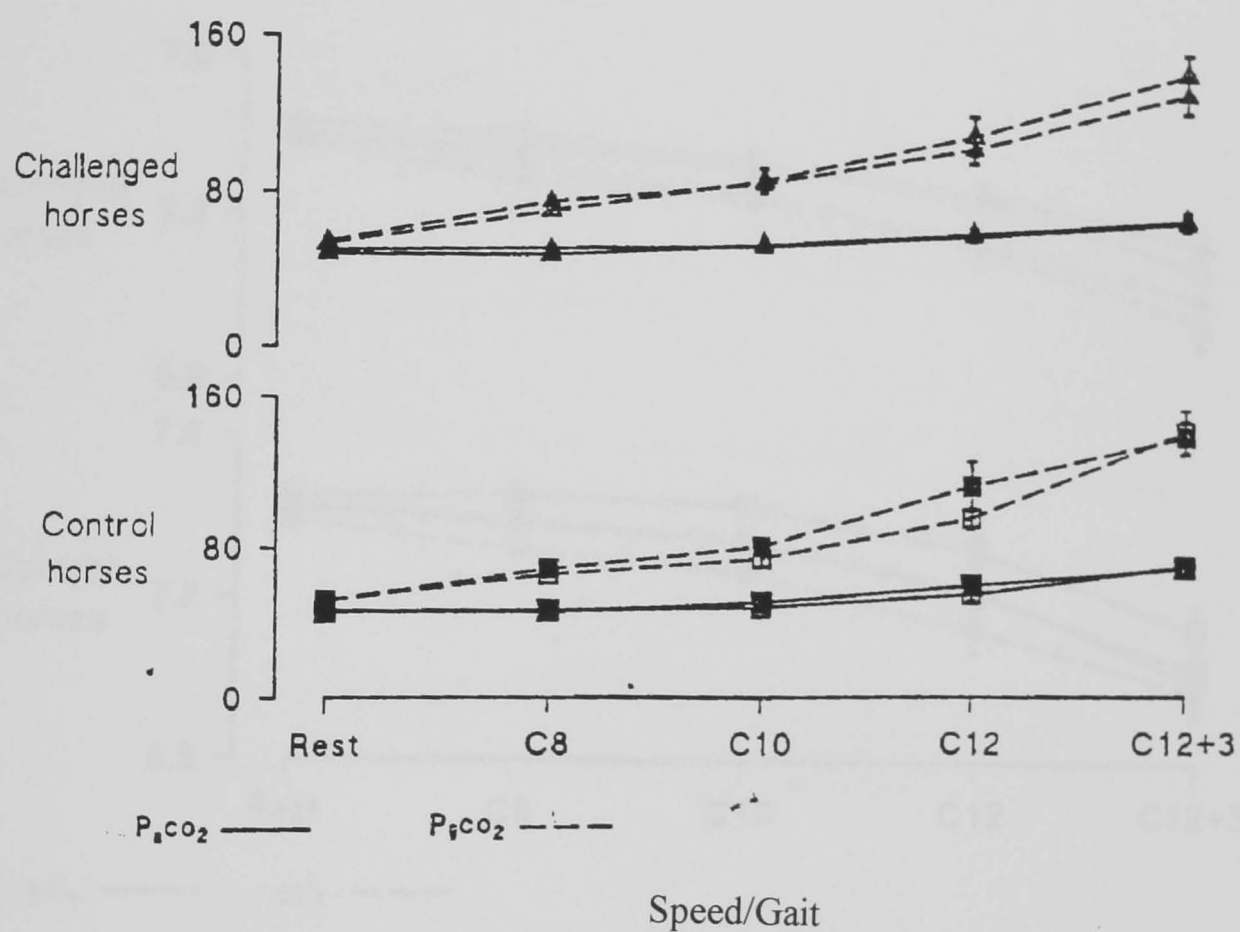


Figure 10.7: Blood pH during exercise in Thoroughbred horses before and 21 days after influenza challenge

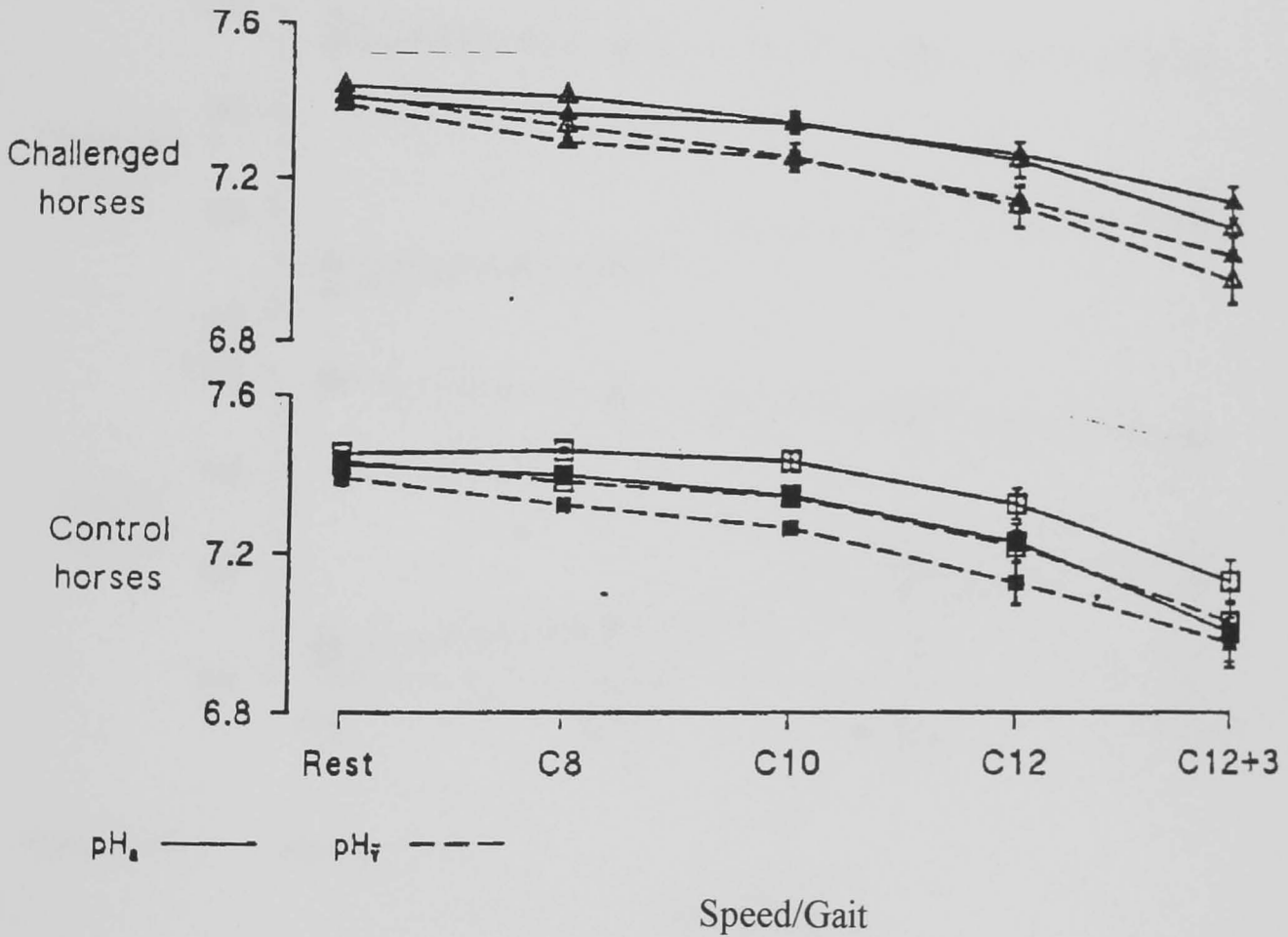
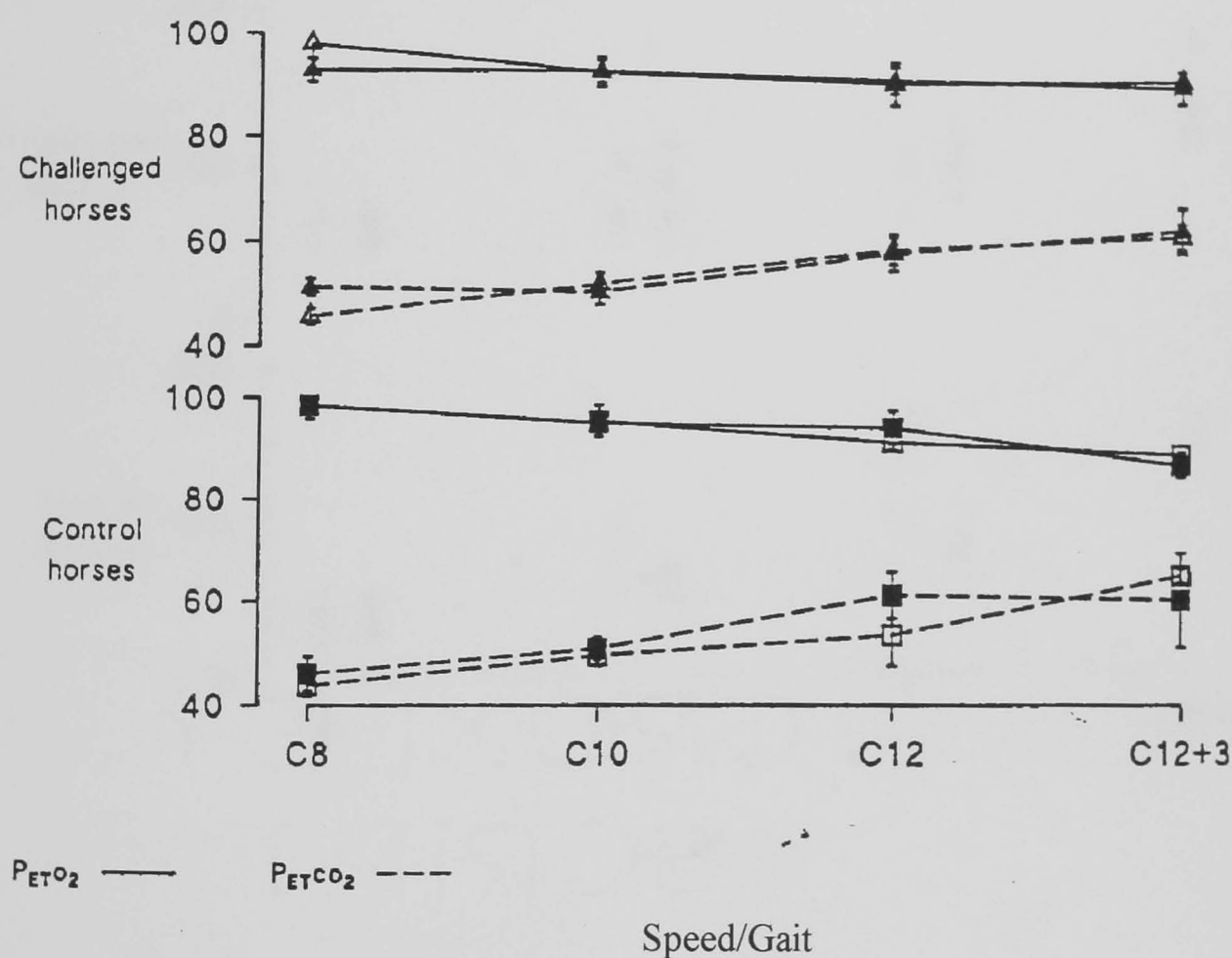
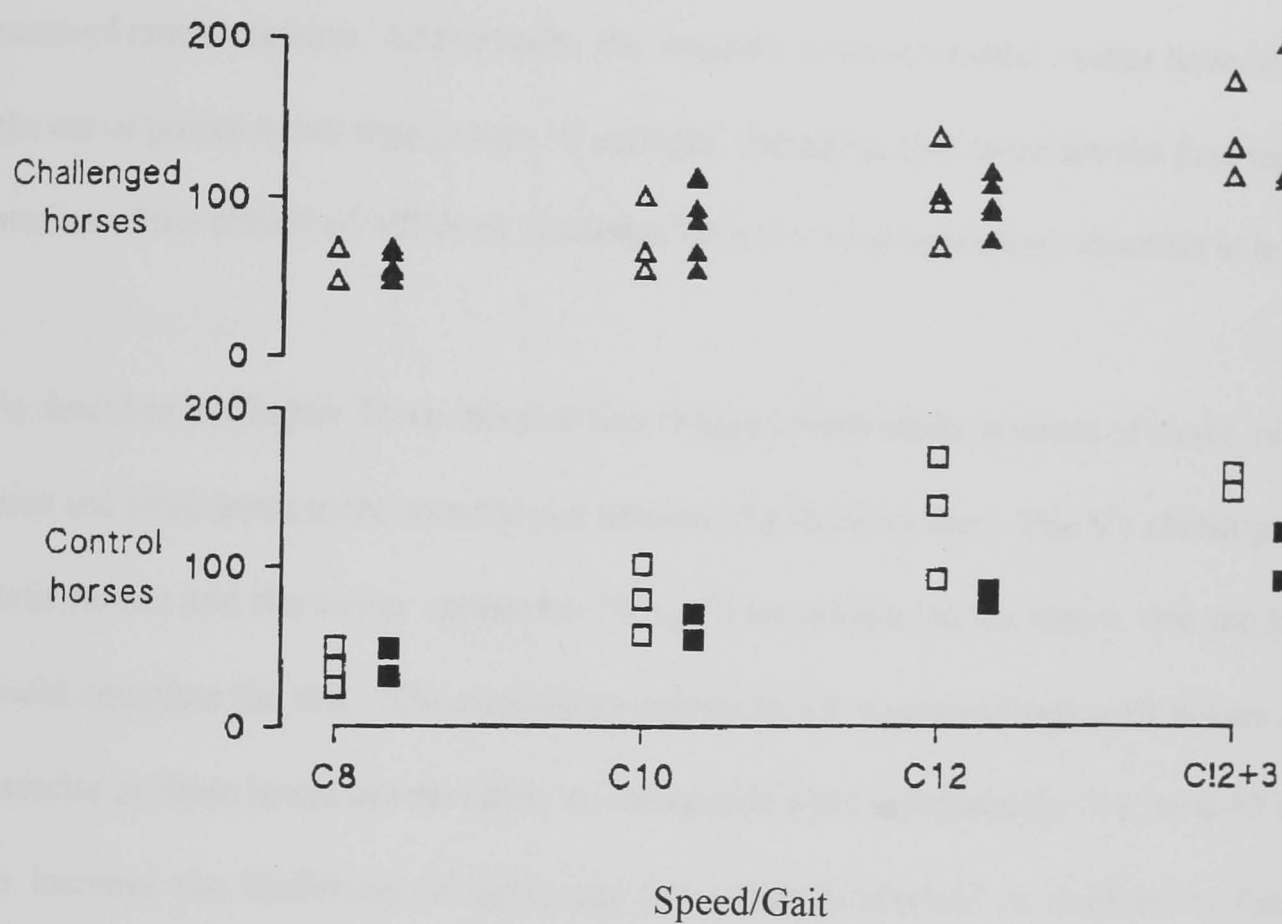


Figure 10.8: End tidal gas tensions (mm Hg) during exercise in Thoroughbred horses before and 21 days after influenza challenge





**Figure 10.9: Oxygen consumption during exercise in Thoroughbred horses before and 21 days after influenza challenge**



*Note to Figure 10.9: Missing data points are, as in previous studies, occasions where the flow transducer and mass spectrometer outputs could not be aligned.*

## Discussion

As in the V1 and V2 tests, no consistent effect of influenza on respiratory function three weeks post-challenge could be detected.

Although there have been many experimental studies on the pathology and immunology of equine influenza, the effects of the disease on respiratory function do not appear to have received much attention. Additionally, the majority of experimental studies have involved the use of ponies rather than horses. It appears, therefore, that these are the first reported studies of the effects of influenza challenge on exercising respiratory function in horses.

As described in Chapter Three, Section (ix) changes were made in terms of strain of virus used and work levels in the exercise test between the three studies. The V1 challenge used unfit horses and the canter speeds ( $6-10 \text{ m.s}^{-1}$ ) were kept low to ensure that the horses could complete the test. The experience gained in V1 suggested that unfit horses could exercise at these levels comfortably, so the speeds were increased for V2 (to  $8-12 \text{ m.s}^{-1}$ ) to increase the likelihood of detecting low-grade alterations in respiratory function following infection. Since the V3 study used fully-trained horses and as the effects of challenge in V1 and V2 were slight, still higher workloads were used ( $8-12 \text{ m.s}^{-1}$  and  $12 \text{ m.s}^{-1}$  @  $3^\circ$  incline) for the final challenge study.

The virus strain was changed from Newmarket/79 in V1 to Suffolk/89 in V2 and V3 as the latter is a more recent strain, not present in the vaccines which these horses had been given and it was felt that it would be more likely to induce clinical signs in partially-immune horses.

In V3, four of the five horses undergoing their first challenge were shown to be infected either by virus isolation, serology or both of these techniques. In one previously unchallenged horse and in the horse being rechallenged (Monty), no infection was demonstrable. This is interesting in the case of Monty since his circulating antibody titres to influenza had fallen to low levels since his previous challenge. It would appear likely that his resistance to infection was due to local antibody production and/or cell mediated immunity both of which have been demonstrated following equine influenza infection in ponies (Hannant *et al.*, 1988b; Hannant *et al.*, 1989; Hannant and Mumford, 1989).

The effects of influenza on respiratory function were mild, the only alterations in measured parameters being a change in the pattern of breathing in two horses, although in one of them (Ross) this was restricted to the low speed canters and one would generally expect changes in respiration of pathological origin to persist if not worsen with increasing workload. The other horse to show a change in breathing pattern (Flighty) showed altered respiratory: locomotory coupling during C8 and C12+3.

The significance of these changes is uncertain since neither horse showed any sign of impairment to gas exchange. Although Ross failed to complete the final canter of his test, care is necessary in interpreting this fact since knowledge of the recent challenge history of the horses may have affected the decision to stop the test and Monty who showed no evidence of infection was also, probably erroneously, stopped before completion of his test. Taking the three challenge studies together, the only other change in measured variables occurred in the V1 challenge when alterations in acid-base balance and blood lactate were observed in Hero; this finding was discussed in the previous chapter. Flighty's loss of

respiratory: locomotory coupling, particularly during C12+3 may indicate a residual effect of influenza infection on respiratory function (this sign was seen in a number of the referred cases which were exercise tested - see Chapter Eleven), however in the absence of other changes in respiratory function tests, this assumption is at present only speculation.

Buddy showed a reduction in the proportion of normal breaths during canter during his *pre*-challenge test. As described earlier, he showed signs of airway inflammation during his control period, but not following infection. It appears likely therefore, that the difference in the pattern of his breathing between the pre- and post-challenge tests (i.e., abnormal before challenge, normal after) reflects recovery from his earlier pulmonary inflammatory condition.

In man, a variety of respiratory viral infections may result in alterations in lower airway function although these may not be noticed due to the more severe upper respiratory tract symptoms (Gibson, 1984). Abnormalities of peripheral airway function are difficult to detect due to the large normal ranges of many pulmonary function tests, but repeat measurements on individuals several weeks after infection may reveal alterations (Gibson, 1984).

Influenza infection may result in mild reductions of lung volumes, a reduction in  $P_aO_2$  and an increase in  $P_{(a-\bar{v})}O_2$  (Johanson, Pierce and Sanford, 1969). Other workers have reported a reduction in steady-state carbon monoxide diffusing capacity ( $D_Lco$ ) for several weeks following presumptive influenza infections (Horner and Gray, 1973). Repeat testing

showed a mild increase in total airways resistance following influenza A infection, although pre-and post-infection values remained within the normal range (Little, Hall, Douglas *et al.*, 1978).

Following 'colds', various subtle changes in lung function have been reported including changes in steady-state  $D_{Lco}$  (Cate, Roberts, Russ and Pierce, 1973), frequency dependence of compliance (Picken, Niewoehner and Chester, 1972; Blair, Greenberg, Stevens *et al.*, 1976) and abnormalities of maximum expiratory flow (Fridy, Ingram, Hierholzer and Coleman, 1974).

In the current studies, eight horses were successfully infected. In just two animals mild changes in breathing pattern were observed post-challenge and in one other there were alterations in acid-base balance and blood lactate concentration. Hence the effects of this level of disease in partially-immune Thoroughbreds, rested after challenge, appears to be generally slight by 21 days post-infection, although individual animals may show evidence of alterations in exercising acid-base balance. Further studies are indicated to determine the acute effects of influenza infection on respiratory function and its effect on unvaccinated animals as well as exploring further the partially-immune animal typical of the British racehorse population.

## **CHAPTER ELEVEN - RESPIRATORY RESPONSES TO EXERCISE IN HORSES REFERRED FOR CLINICAL INVESTIGATION**

The respiratory response of normal Thoroughbred horses to a standardised exercise test has been described (Chapters Six and Seven) and the influence of training on this has been considered (Chapter Eight). This chapter is concerned with the application of exercise testing to horses referred for investigation of performance-related problems, with a view to evaluating the clinical usefulness of this procedure.

### **Summary of Methods**

All cases were referred by veterinarians in practice. To enable a comparison to be made with data from experimental horses, only Thoroughbred horses were included.

Following admission, referred horses underwent a routine evaluation to exclude horses with orthopaedic conditions which would render them liable to injury during treadmill exercise. The horses then underwent a period of familiarisation to the treadmill over a few days whilst other diagnostic tests were performed (clinical examination at rest, routine haematology, endoscopy of the respiratory tract and tracheal wash aspiration).

When suitably accustomed to treadmill exercise, the horses performed an exercise test similar to the one used in the previously described studies, the canter speeds used being 8, 10 and 12 m.sec<sup>-1</sup> on a level surface (C8, C10 and C12 respectively). For a fuller description of the methods see Chapter Three.

Data from exercise tests on referred horses were compared with values from the fully-fit, clinically normal Thoroughbreds (see Chapter Seven). Data from horses suspected of suffering from respiratory disease were compared with those from the control group using an unpaired Student's *t* test, the level of significance being taken as  $p < 0.05$ . As is normal for the formulation of a reference range for clinical testing, the reference range for individual comparisons was taken to be between two standard deviations above and below the mean of the normal animals.

## **Results**

Thirteen horses were exercise tested, several others being excluded, mostly due to orthopaedic conditions, one was excluded because upon arrival he was found to show signs of overt respiratory disease and a tracheal wash examination revealed the presence of severe, acute airway inflammation. The age, sex and reason for referral of the selected horses tested are shown in Table 11.1. All horses were Thoroughbred horses in race training. All of the animals tested adapted well to treadmill exercise over a period of a few days.

### *Other diagnostic procedures*

Since it was necessary to use whatever clinical cases could be obtained by referral from practicing veterinarians, the group of selected, referred horses represents a relatively heterogeneous group of animals in terms of their exact clinical signs and the causes of them. It is appropriate, therefore, before considering exercise test results to describe briefly the results of other diagnostic procedures which were carried out (in particular endoscopic examination of the respiratory tract), and how the animals were then

categorised for interpretation of exercise testing results.

A resting clinical evaluation of all cases revealed no significant signs of disease, except for one horse, Case 2, in whom a slight, serous nasal discharge was noted occasionally. Two horses, Cases 5 and 11 came from stables in which it was later reported that unspecified respiratory infections had been diagnosed shortly prior to referral. Routine haematology and biochemistry were generally unremarkable, although one horse (Case 6) had a slight elevation of serum gamma glutamyl transferase activity ( $59 \text{ iu.l}^{-1}$ , normal range: 0-53), this is a common finding in horses in training.

When exercised outdoors, either ridden or on the lunge, abnormal respiratory sounds were audible in four horses. In three cases, 8, 10, and 11 the abnormal sound was inspiratory. The sound consisted of a whistling sound for Case 8, a harsh rattle for Case 10, and a low-grade inspiratory sound for Case 11. Case 9 made excessively loud inspiratory and expiratory sounds during exercise.

Three horses displayed very mild signs of lameness: Case 10 showed intermittent right hindleg lameness, Case 11 and 13 bilateral forelimb lameness, and Case 9 a hind limb assymetry suggestive of low-grade sacro-iliac ligament damage. In no cases were the referring veterinarian or the trainer aware of the lameness and it was not considered likely to be worsened by treadmill exercise.



**Table 11.1: Details of referred horses**

<i>Horse</i>	<i>Age</i>	<i>Sex</i>	<i>Reason for referral</i>
1	3	c	poor performance occasional abnormal sounds during races
2	10	g	poor performance
3	5	g	poor performance
4	7	g	poor performance
5	3	c	poor performance
6	6	g	poor performance
7	7	g	poor performance
8	5	g	poor performance/ abnormal sounds during exercise
9	5	g	poor performance
10	5	g	poor performance
11	3	c	poor performance
12	4	g	abnormal post-race behaviour
13	5	g	post-race collapse

c - colt    g - gelding

Resting endoscopic examinations of the upper respiratory tract were remarkable in only three cases. One animal, Case 8, showed moderate laryngeal asymmetry at rest, to a degree which was graded as grade II/IV on the scale used by Hackett, Ducharme, Fubini and Erb (1991).

In another horse, Case 9, it was impossible to pass the 13mm diameter endoscope routinely used for examination and only with a smaller endoscope could the pharynx be reached (the referring veterinarian had also reported that he was unable to pass his endoscope into the pharynx). Radiographs of the head of this horse revealed no abnormalities of structure but the nasal chambers did subjectively appear narrower than expected for a horse of this size (this observation was made 'blind' by a colleague with no prior knowledge of the history of the horse).

Case 10 was examined several times endoscopically at rest and for the majority of the time, his epiglottis was displaced dorsally to the soft palate, the tip of the epiglottis sometimes being just visible for a part of the respiratory cycle behind the caudal border of the soft palate. When the epiglottis did assume a 'normal' position, dorsal to the soft palate, it was frequently entrapped in a fold of aryepiglottic mucous membrane and was rarely seen in the normal, unentrapped position. A small nodule was visible on the left arytenoid cartilage. This horse was reported to have undergone a soft palate resection the previous year. Although it is not usually considered that dorsal displacement of the soft palate at rest is diagnostic for the occurrence of this condition during exercise, the degree of abnormality in this horse's pharynx suggested that it was extremely likely to occur during fast exercise.

Exercising videoendoscopy was possible in three cases, this was performed on a separate occasion from the standardised exercise test. In one horse, Case 1, no abnormalities were noted. Dynamic collapse of the left side of the larynx typical of recurrent laryngeal neuropathy was observed in Case 8 and Case 11 was found to have dorsal displacement of the soft palate for long periods during exercise at 12 m.s<sup>-1</sup> on a 5° incline.

### *Tracheal wash findings*

Tracheal wash samples were obtained endoscopically from all horses, using the technique described by Whitwell and Greet (1984). The results of these examinations are summarised in Table 11.2. Three horses (Cases 3, 6 and 7) had an inflammation score which would be significant by the criteria of Whitwell and Greet (1984), whilst a further five (Cases 1, 2, 4, 5, and 9) had a score of 1/3.

### *Grouping of referred horses*

On the basis of reason for referral, endoscopy results and tracheal wash inflammation score, the thirteen horses were grouped as follows:

- Group A:* Referred for suspected respiratory disease; inflammation score greater than zero; no upper airway abnormalities observed.
- Group B:* Referred for suspected respiratory disease; inflammation score 0-1; evidence of upper airway abnormality.
- Group C:* Referred for abnormal post-race behaviour (distress, collapse); inflammation score zero; no upper airway abnormality observed.

The horses were therefore divided as follows:

*Group A:* Cases 1, 2, 3, 4, 5, 6, and 7

*Group B:* Cases 8, 9, 10, and 11

*Group C:* Cases 12 and 13

### *Exercise Testing*

All of the horses were able to complete the exercise test. The individual breaths taken by the horses during the canters were assessed and graded as in Chapter Six. Table 11.3 shows the proportion of normal breath types occurring in each canter for each horse.

In seven animals the percentage of normal breaths increased appreciably with speed until during C12, they took over 94% normal breaths. In the clinically-normal horses no significant change occurred in percentage of normal breaths as speed increased<sup>1</sup>. Pathological alterations in the pattern of breathing might reasonably be expected to remain consistent in proportion or increase with workload. Hence it appears that although all horses had undergone several days treadmill training, with at least two practise runs wearing the mask, it may be that at the lower speeds, psychological factors play a role in determining the pattern of breathing in some horses which is obviated at higher workloads when the metabolic demands of exercise become overriding. For this reason,

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<sup>1</sup>Mean percentages of normal breaths at each speed for the clinically-normal horses were:

C8	-	93.7% (reference range: 83.5-100.0)
C10	-	95.6% (reference range: 88.2-100.0)
C12	-	97.6% (reference range: 95.6-99.4)

**Table 11.2: Summary of tracheal wash results from referred horses**

<i>Horse</i>	<i>Neutrophil proportion*</i>	<i>Nucleated cell count</i>	<i>Inflammation Score¶</i>
1	+	+	1
2	++		1
3	+++	+	2
4	+	+	1
5	+		1
6	+++	+	3
7	++	++	3
8	+		0
9	+++		1
10	+		0
11	+		0
12	+		0
13	±		0

\* - scored from 1-3, 0-1 is taken as normal

¶ - scored from 1-3. One point each for: (i) excess tracheal mucus, (ii) ++ or +++ neutrophils, (iii) elevated nucleated cell count ( $>1000\text{ cmm.}^{-1}$ ). Whitwell and Greet (1984) suggested that a score of more than one is suggestive of significant airway inflammation.

the highest speed canter (C12) is used for comparison of the referred horses with the reference range. Table 11.4 shows values for respiratory variables during C12 in the referred horses, with the reference ranges for comparison, Table 11.5 summarises these changes qualitatively for easy reference, whilst Figures 11.1-11.6 show the distribution of some variables graphically.

When the horses referred for investigation of respiratory disease (Groups A and B) were compared as a single group to the control horses significant differences between the groups were seen for  $f_b$ ,  $\dot{V}_e$ , PEF,  $t_i$  and  $t_i/t_e$ . Individual data are now considered.

Arterial blood gases<sup>2</sup> were not indicative of impaired gas exchange, indeed for five horses (four in Group A and one in Group C)  $P_aO_2$  was *above* the reference range during C12, and in one horse (Case 5, Group A)  $P_aCO_2$  was slightly low.  $C_aO_2$  was slightly lower than the reference range for Cases 4 and 11, in both of whom [Hb] was low during C12.

Since these horses were referred from practitioners, it was not always acceptable to use a mixed venous catheter, hence mixed venous blood gas data are available for only six horses. In horses in which  $t_{\bar{v}}$  was not measured, due to the lack of a mixed venous catheter, the mean value for  $t_{\bar{v}}$  for the horses in which it was measured was used.  $pH_a$ ,  $P_{ET}O_2$ ,  $P_{ET}CO_2$ , mixed venous blood gases and  $pH_{\bar{v}}$  were always within the reference ranges during C12.

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<sup>2</sup>In one horse, Case 8, arterial blood gas data were not available due to the arterial catheter becoming damaged early during the test.

**Table 11.3: Percentage of normal breaths taken during canter by the referred horses.**

<i>Group Horse</i>		<i>C8</i>	<i>C10</i>	<i>C12</i>
A	1	*	<b>61.5</b>	<b>56.2</b>
	2	98.0	98.0	96.2
	3	88.5	90.4	98.7
	4	<b>79.1</b>	93.8	<b>94.6</b>
	5	<b>24.6</b>	96.9	97.6
	6	91.7	98.7	98.7
	7	85.6	95.7	<b>89.4</b>
B	8	<b>58.9</b>	<b>87.6</b>	98.3
	9	96.8	<b>66.9</b>	<b>65.9</b>
	10	83.7	<b>83.7</b>	<b>74.9</b>
	11	<b>62.1</b>	94.1	98.1
C	12	96.1	97.2	97.0
	13	88.2	95.6	98.2

Figures in **bold** indicate canter with percentage of normal breaths less than the reference range.

\* - trace unreadable due to chart recorder failure

**Table 11.4: Comparison of values for some respiratory variables during exercise in clinically normal Thoroughbred horses and referred animals**

	Reference range	1	2	3	4	5	6	7	8	9	10	11	12	13
$f_b$	111.8-131.8 (121.8)	124.1		131.6	119.2	117.2		114.1	<b>73.1</b>	118.6	<b>107.9</b>		121.5	<b>87.7</b>
$V_t$	9.6-20.4 (15.0)	11.0		<b>9.5</b>	11.7	9.8		11.9	16.7	12.3	10.4		11.7	15.5
$\dot{V}_e$	1213.1-2419.9 (1816.5)	1367		1249	1398	<b>1149</b>		1362	1221	1464	<b>1127</b>		1424	1355
$t_i$	0.222-0.262 (0.242)	0.230		0.230	0.248	0.260		0.256	<u>0.440</u>	<u>0.274</u>	<u>0.317</u>		0.256	<u>0.463</u>
$t_e$	0.222-0.282 (0.252)	0.260		0.226	0.256	0.252		0.270	<u>0.420</u>	0.232	0.282		0.238	<u>0.416</u>
$t_i/t_e$	0.828-1.096 (0.962)	0.890		1.018	0.969	1.032		0.948	1.090	<u>1.182</u>	<u>1.124</u>		1.076	<u>1.115</u>
PIF	47.6-74.0 (60.8)	<u>78.0</u>		52.1	55.4	51.6		<u>75.9</u>	50.8	68.7	<b>45.8</b>		57.1	<b>35.2</b>
PEF	63.3-100.1 (81.7)	65.7		<b>58.2</b>	<b>60.0</b>	<b>54.8</b>		65.2	74.2	71.4	<b>52.6</b>		67.4	<b>61.3</b>
$P_aO_2$	53.1-80.3 (66.7)	<u>96.5</u>	67.6	76.3	<u>82.9</u>	<u>91.3</u>	<u>93.0</u>		59.1	74.6	72.0	<u>81.0</u>		66.5
$P_aCO_2$	45.9-67.1 (56.5)	47.0	58.1	53.6	<b>45.1</b>	51.2	48.0	49.0	58.7	46.6	53.3	53.7		56.1
$pH_a$	7.082-7.522 (7.302)	7.358	7.342	7.365	7.298	7.207	7.394	7.350	7.328	7.430	7.365	7.329		7.387
$p\bar{v}O_2$	11.5-23.5 (17.5)	18.8		18.6	17.0				16.7	15.0	16.0			
$p\bar{v}CO_2$	57.4-149.8 (103.6)	84.6		83.1	70.5				92.8	76.4	85.6			
$pH_{\bar{v}}$	6.931-7.447 (7.189)	7.244		7.278	7.235				7.241	7.322	7.274			

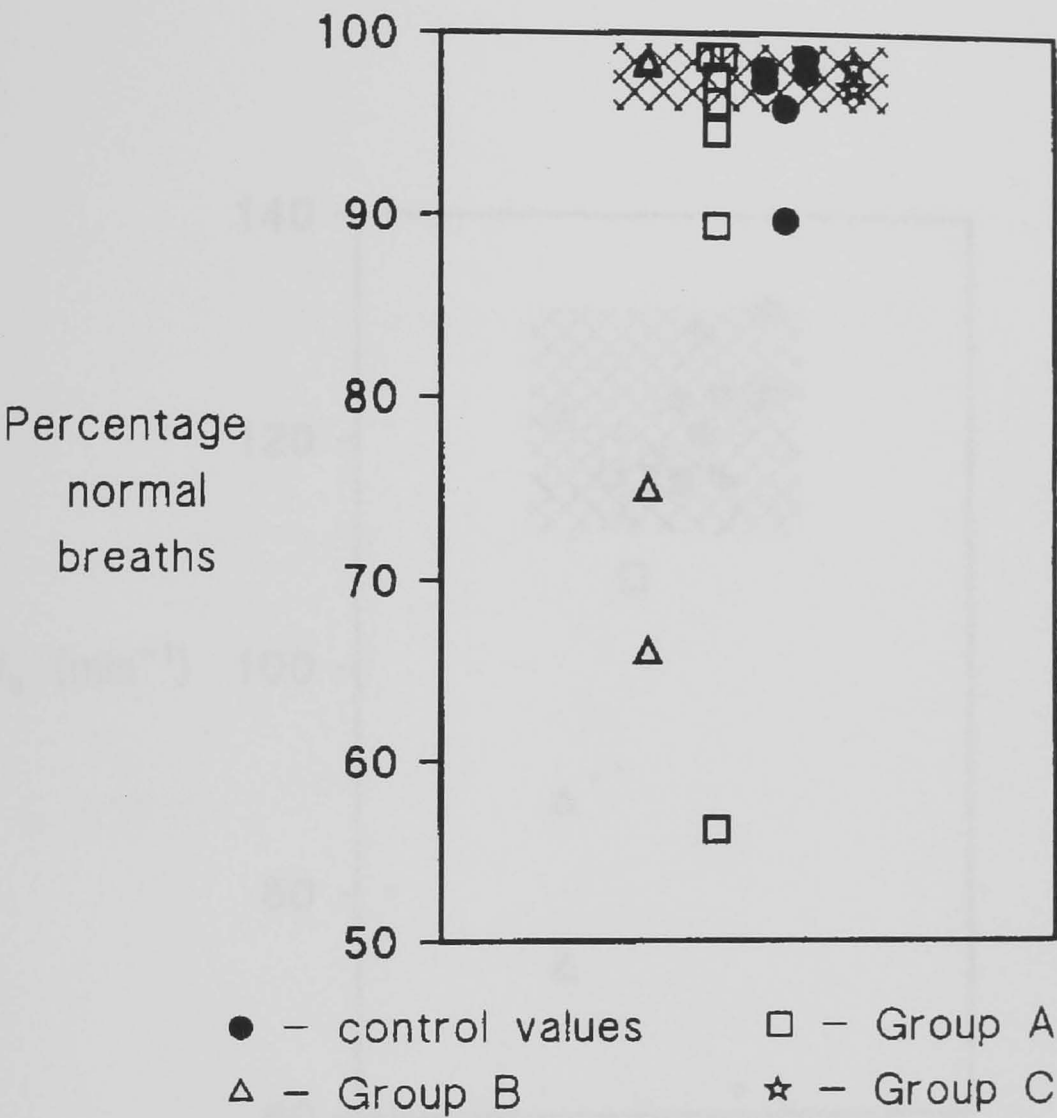


**Table 11.5: Variations from reference values of respiratory parameters in referred during exercise at 12 m.s<sup>-1</sup>**

Case no.	f <sub>b</sub>	V <sub>t</sub>	V̇ <sub>e</sub>	PIF	PEF	t <sub>i</sub>	t <sub>e</sub>	t <sub>i</sub> /t <sub>e</sub>	P <sub>a</sub> O <sub>2</sub>	P <sub>a</sub> CO <sub>2</sub>	[Hb]	
Group A												
1									↑		1	
2									↑		2	
3				↑							3	
4						↑		↑			↓	4
5					↓				↑	sl↓		5
6			↓		↓				↑			6
7	↓		↓	↓	↓	↑		↑				7
Group B												
8									nd	nd		8
9												9
10	↓					↑	↑					10
11	↓			↓	↓	↑	↑	↑			↓	11
Group C												
12				↑					↑			12
13		↓			↓							13

↑ = value greater than reference range  
↓ = value lower than reference range  
sl = slight variation only  
nd = not done

**Figure 11.1: Comparison of proportion of normal breaths during C12 for referred horses with control values**



*In figures 11.1-11.6 the hatched area represents the reference range (mean  $\pm$  2SD for normal Thoroughbreds in Chapter Seven)*

Figure 11.2: Comparison of  $f_b$  during C12 for referred horses with control values

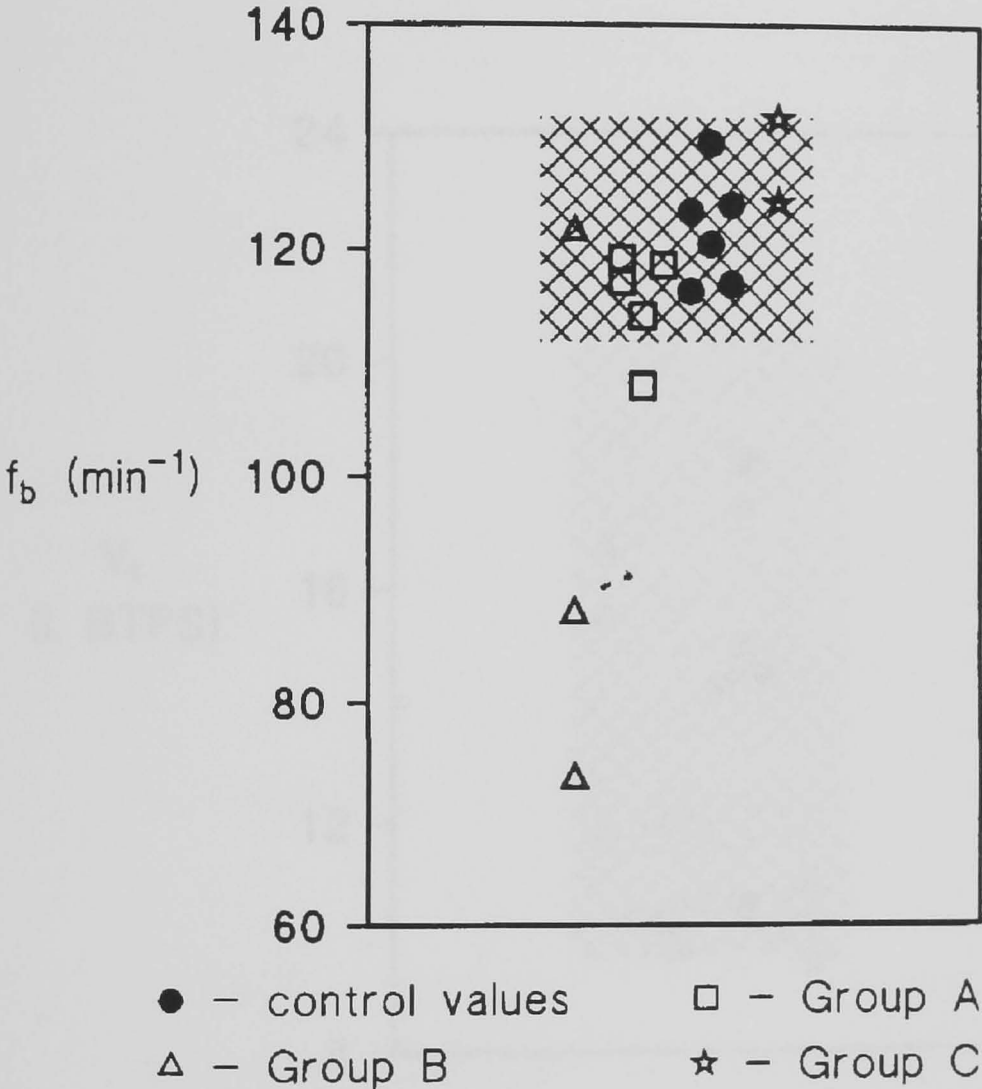


Figure 11.3: Comparison of  $V_t$  during C12 for referred horses with control values

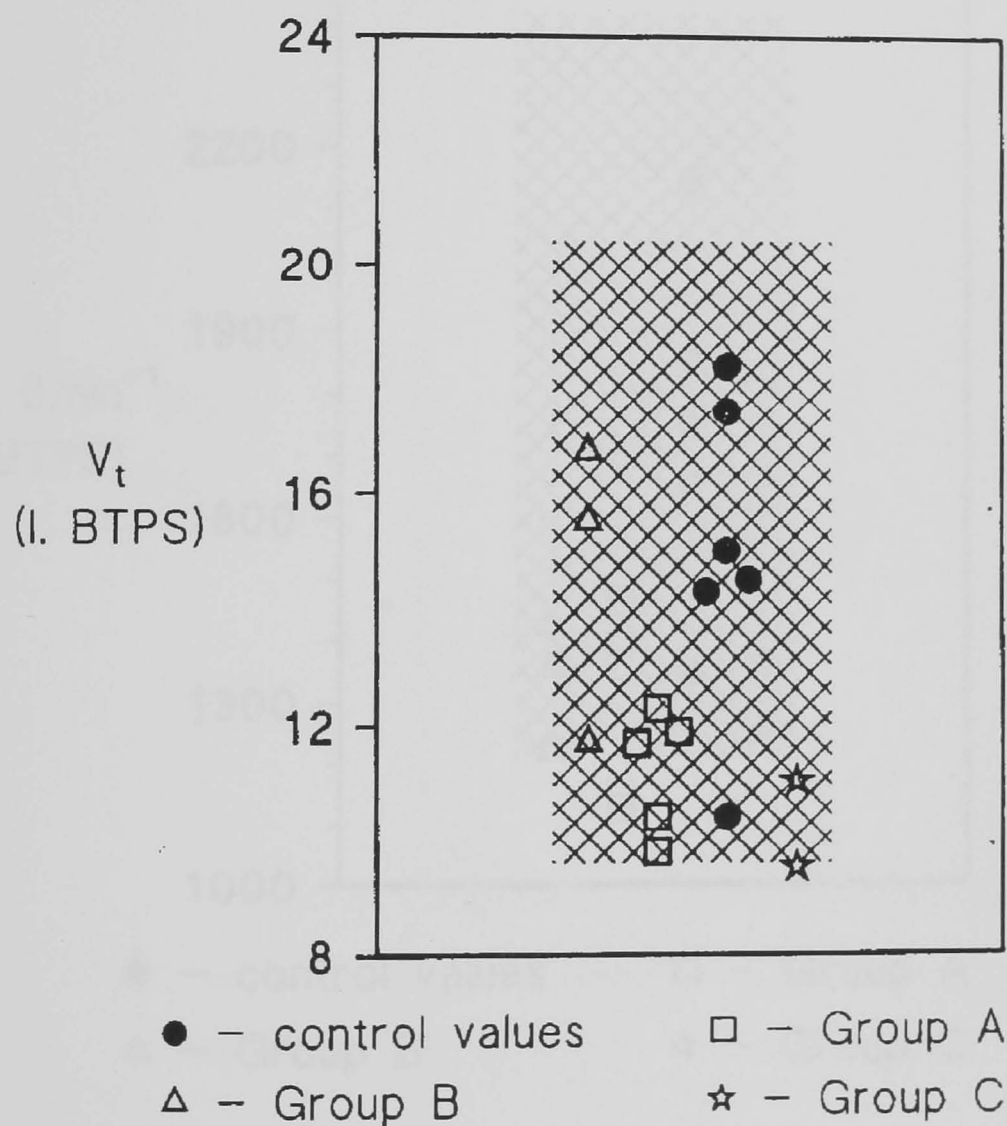


Figure 11.4: Comparison of minute volume during C12 for referred horses with control values

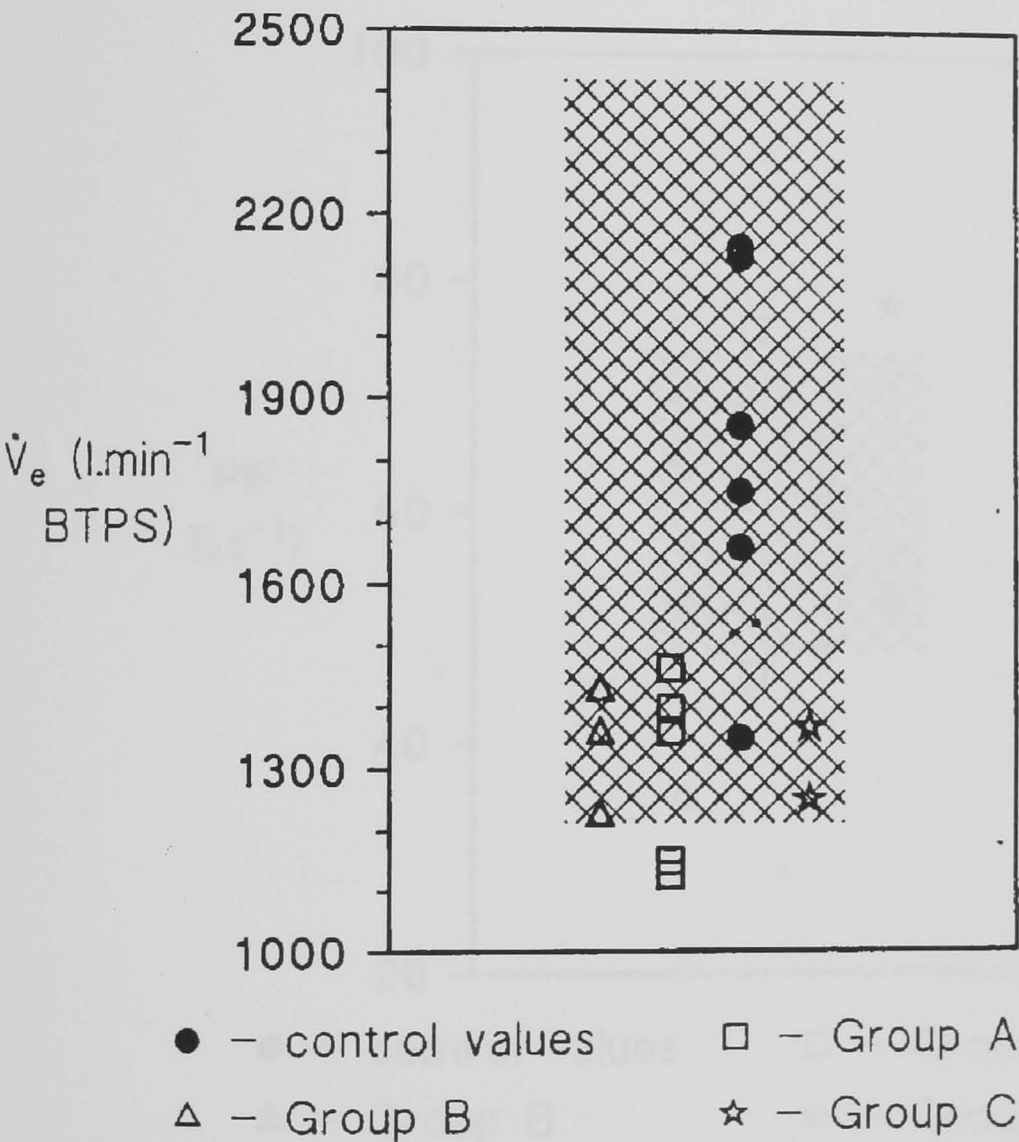
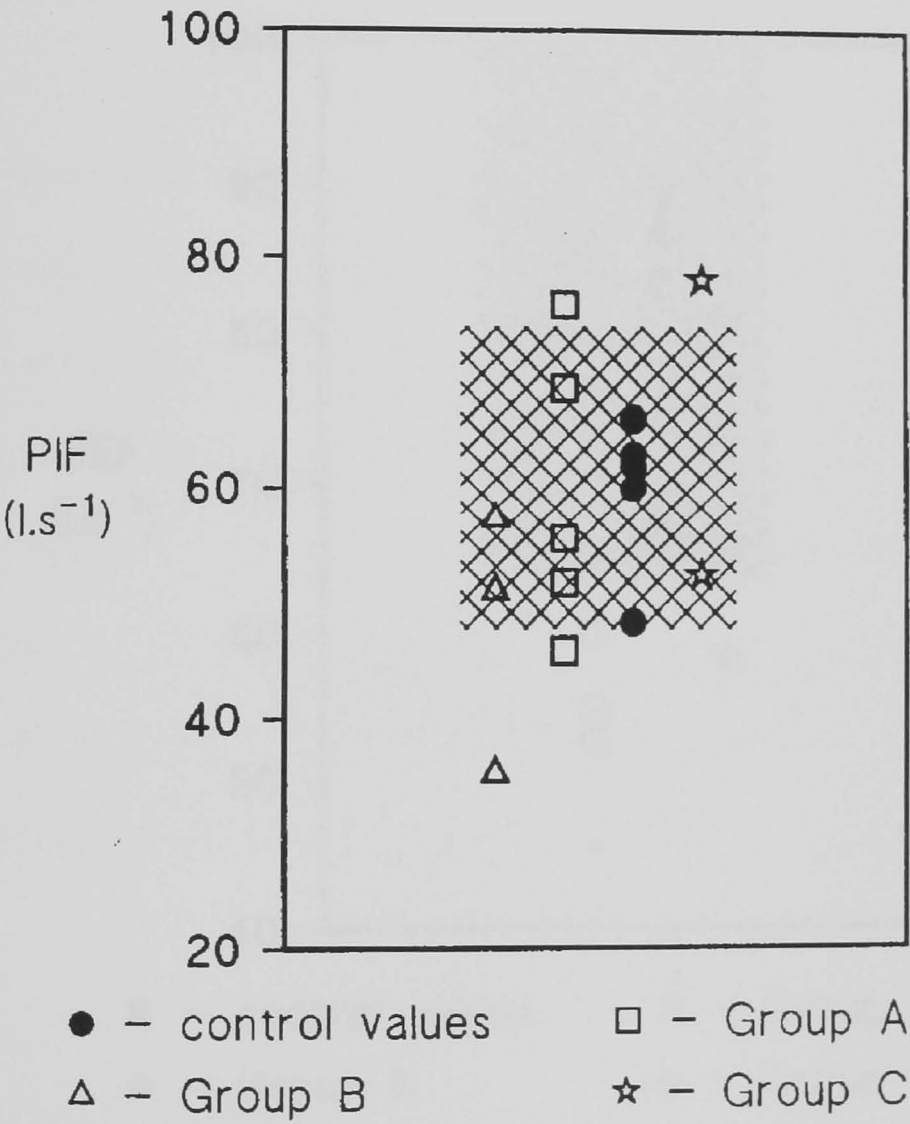


Figure 11.5: Comparison of PIF during C12 for referred horses with control values





Further results are now considered by clinical grouping.

### *Group A horses*

This group, comprising the horses referred with suspected respiratory disease, with positive inflammation scores and no indication of lower airway disease, showed a varied response to exercise.

In two horses (Cases 1 and 2), ventilatory parameters could not be measured during C12 due to equipment problems during the latter part of the test. Of these two, Case 2 showed a normal pattern of breathing with 96.2 % of breaths being normal. Case 1 however, had an abnormal pattern of breathing in which 56.2% of breaths were normal, but 41.8% of breaths were biphasic inspirations and represented long periods of 1:2 respiratory:locomotory synchrony (during C10, he showed a similar pattern, albeit for a shorter period of respiration - 36.8% of breaths being biphasic inspirations with 1:2 synchrony at this speed). Despite this pattern of breathing,  $P_{aO_2}$  was higher than the reference range.

Two of the other horses showed little variation from the reference range during C12. Case 3 showed a slight increase in PIF whilst  $t_i$  and  $t_i/t_e$  were both high for Case 4. Both horses showed a normal pattern of breathing with 98.7% and 94.6% normal breaths respectively.

The remaining three horses (Cases 5, 6 and 7) showed a reduction in PEF during C12. In one of these, Case 5,  $V_t$  and  $\dot{V}_e$  were maintained despite this and the pattern of breathing remained normal. For Case 6  $V_t$  was towards the lower end of the reference range and  $\dot{V}_e$



was reduced. Case 7 showed a reduction in PIF, PEF and  $\dot{V}_e$ , with an increase in  $t_i$  and  $t_i/t_e$ . This horse also showed a reduction in  $f_b$  which was due to an increase in the proportion of double inspiratory breaths to 9%, resulting in the horse varying between 1:1 and 1:2 respiratory:locomotory synchrony.

### *Group B Horses*

This group, the horses with upper airway obstructions, also showed some variation in response. Case 8 showed no abnormalities of ventilation and a normal pattern of breathing.

For Case 9 ventilatory data were not available during C12, again due to equipment failure, but the pattern of breathing was altered during both C10 and C12. During C12, only 65.9% of breaths were normal in form, the remainder consisting of 24.1% double inspirations and 9.4% other abnormal breaths representing a loss of 1:1 respiratory:locomotory synchrony for approximately a third of the canter.

Case 10 also showed a significant alteration in the pattern of breathing. Only 74.9% of breaths were normal and these were interspersed with 4.8% double inspirations and 16.2% slow breaths taking two strides instead of one<sup>3</sup>. Hence respiratory:locomotory synchrony was 1:2 rather than 1:1 for a fifth of the canter.  $f_b$  was reduced and  $t_i$  and  $t_e$  increased. Although  $\dot{V}_e$  was within the reference range it was towards the lower end of the range (1,221l; reference range 1,213.1-2,419.9l).

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<sup>3</sup>3% of breaths during this canter were small breaths taken at the start of the canter.

Case 11 showed a reduction in  $f_b$ , PIF and PEF, with an increase in  $t_i$ ,  $t_e$  and  $t_i/t_e$ . Individual breaths were normal in form, although peak flows were noted to vary more on a breath-by-breath basis than is usually the case.

### *Group C Horses*

These two horses (the ones referred for investigation of post-race abnormalities) both showed a normal pattern of breathing. Case 12 showed no sign of impaired ventilation, in fact PIF was higher than the reference range. Case 13 showed a reduction in PEF and a slight reduction in  $V_t$  during C12.  $\dot{V}_e$  was towards the lower end of the reference range (1,249l; reference range 1,213.1-2,419.9l).

### **Discussion**

The test proved to be easily performed by all horses and no animals showed any ill-effects afterwards. This is obviously of importance if a test is to be acceptable as a diagnostic procedure.

Measurement of  $P_{aO_2}$  and maximum change in pleural pressure during breathing have been used as pulmonary function tests in the resting horse (Macpherson *et al.*, 1982; Derksen, 1991) in the diagnosis of COPD. Unfortunately, these parameters are generally only altered when the severity of disease is such that it may be readily diagnosed by a diligent clinical examination. Since the stress of exercise may reveal abnormalities of respiratory function when resting pulmonary function tests lie within the normal range (Denison *et al.* 1984; West, 1992), exercise testing may aid the detection of horses with sub-clinical respiratory disease. Additionally, the measurement of pulmonary function during exercise

may assist in the assessment of the functional significance of observed clinical abnormalities as well as providing objective information concerning the effect of treatment. The latter point may be of particular importance in the assessment of laryngeal function, since asynchronous movement of the larynx at rest is common (Baker, 1983) but may not reflect the functional abilities of the larynx during exercise.

Unfortunately, as was noted in Chapter 10, many pulmonary function tests have a wide normal range (Gibson, 1984) and this may impede the detection of low-grade changes in function on one-off examinations. This is notable in the reference range of data used in these studies (see Table 11.4).  $P_{aO_2}$  and  $V_t$  for example both show a wide range (53.1-80.3 mm Hg and 9.6-20.4l respectively). This may be in part due to the small sample size and with a larger group to provide a reference range, these ranges may reduce slightly, however it is likely that considerable variation exists between individuals in the response to exercise, as is the case with certain other physiological variables during exercise, e.g. blood lactate concentration. For variables in which this is true, one-off tests are of limited value and serial testing is necessary to obtain useful information.

Large between individual variation may explain the lack of blood gas abnormalities during exercise in the referred horses, although of course, it is possible that the degree of respiratory dysfunction present was simply insufficient to impair gas exchange measurably at the level of exercise used here. If the latter is the case it may be that either the respiratory disease detected is not significant or that the horse is able to compensate for it at the workloads used in this test (but possibly not at the higher workloads of racing). Maintenance of adequate ventilation and gas exchange may be at the cost of an increased

work of breathing. There is evidence that the mechanics of breathing may be a limiting factor to the increase of ventilation during exercise (Art *et al.*, 1990). Hence, any factor which further increases the work of breathing during exercise may be of great significance in a performance horse.

Since there was evidence that the pattern of breathing improved with successive canters at increasing workloads (Table 11.3), it may be that under the artificial conditions of treadmill exercise with the use of a facemask, the most reliable measurements are obtained at relatively high workloads when the metabolic demands of exercise override psychological factors in determining the pattern of breathing.

The group of referred animals described above represent a heterogeneous group of animals with a variety of clinical signs. Nevertheless, they may be divided into three groups based upon their history and the results of clinical examination.

Three of the Group A horses for whom full data were available showed a reduction in PEF during C12, in two cases accompanied by a reduced  $\dot{V}_e$ , in one of which PIF was also low and the pattern of breathing (evidenced by  $f_b$ ,  $t_b$ ,  $t_i/t_e$  and percentage of normal breaths) abnormal. One other horse showed an abnormal breathing pattern and another, increases in  $t_i$  and  $t_i/t_e$ . In two cases, one of which full data were available for, no impairment to respiration was evident.

The two horses in whom  $\dot{V}_e$  was reduced had the highest inflammation scores (3/3). Although the numbers involved are too small to draw firm conclusions, one may

summarise that horses with high inflammation scores show evidence of ventilatory impairment on this test, but horses with more low-grade inflammation (as evidenced by inflammation score) do not necessarily show abnormalities of ventilation. Overall the variables which showed evidence of impaired respiratory function during C12 in Group A most frequently were PEF (three out of five horses for which data were available) and proportion of abnormal breaths throughout canter (three out of seven). In total, abnormalities were noted in five out of seven horses.

Measurement of the work of breathing during exercise in horses with lower airway inflammation but no impairment to ventilation might be a way to determine whether respiratory function is truly unaffected or whether these animals are compensating for the presence of disease in a way that impairs performance by increasing the energetic cost of breathing.

The Group B horses represented a mixture of different causes of upper airway obstruction. In Case 8, endoscopic examination indicated the presence of idiopathic laryngeal hemiplegia (ILH). This condition, involving a variable degree of paralysis of the left side of the larynx due to degenerative changes in the left recurrent laryngeal nerve, has been estimated to occur in 2.6-8.3% of Thoroughbreds (Pascoe, Ferraro, Cannon, Arthur and Wheat, 1981; Raphael, 1982; Baker, 1983; Hillidge, 1986). It is associated with inspiratory stridor and reduced exercise tolerance. Recently the use of exercising videoendoscopy to observe the larynx during exercise (Stick, Derksen, Nickels, Brown, Arden and Fulton, 1990; Morris, 1991) has indicated that in severely affected animals the glottis may become reduced in size to a slit-like opening during inspiration due to collapse

of the left side of the larynx which is drawn across the airway by the negative pressure of inspiration. Many horses however, including Case 8, are affected to a considerably less degree, in these cases the reduction in glottic size during exercise although demonstrable by videoendoscopy is considerably less.

Previous controlled studies of the effects of ILH on respiratory function have usually involved the use of animals in which the condition was experimentally-induced by left recurrent laryngeal neurectomy, a condition which results in total paralysis of the left side of the larynx (Derksen, Stick, Scott, Robinson and Slocombe, 1986; Shappell, Derksen, Stick and Robinson, 1988; Williams, Pascoe, Meagher and Hornof, 1990). When such animals were exercised on a treadmill at low intensity (up to  $4.3 \text{ m.s}^{-1}$  on a  $6.38^\circ$  incline) the condition did not affect  $f_b$ , PEF or expiratory resistance, but did result in inspiratory flow limitation when PIF reached  $25 \text{ l.s}^{-1}$  and increased inspiratory resistance (Derksen *et al.*, 1986). At higher speeds ( $7.2 \text{ m.s}^{-1}$  on a  $6.38^\circ$  incline), neurectomised horses showed increases in inspiratory impedance, inspiratory transupper airway pressure and  $P_a\text{CO}_2$ , as well as decreases in PIF and  $f_b$  (Shappell *et al.*, 1988).

Williams *et al.* (1990) reported an increase in peak inspiratory and expiratory pressures and a decrease in  $f_b$  and maximum speed during maximal ridden exercise in neurectomised horses.

There are few studies of the effects of ILH on respiratory function in naturally-occurring cases of the condition. Dixon (1982) reported arterial hypoxaemia immediately (<20s) following twenty minutes cantering exercise in a group of twenty horses with

naturally-occurring ILH but not in a group of control horses. Seventeen of the twenty were graded as moderately, severely or, in two cases, bilaterally affected. In a case of naturally-occurring total left-sided laryngeal paralysis, Bayly, Grant and Modransky (1984) found  $P_{aO_2}$  to be much lower and  $P_{aCO_2}$  to be much higher during maximal ridden exercise before as compared to following corrective laryngoplasty.

King, Evans and Rose (1994) performed an incremental treadmill exercise test (up to 12 m.s<sup>-1</sup> on a 10% incline) on a group of nine horses with naturally-occurring ILH and reported reductions in peak  $\dot{V}O_2$  and  $P_{aCO_2}$  at 10 m.s<sup>-1</sup>, but no difference in  $P_{aO_2}$  at 10 m.s<sup>-1</sup>, compared with a control group. All of the affected animals in this study were grade III or IV on the laryngeal grading system used by Hackett *et al.* (1991), i.e. the two most severe grades. The case of ILH in the present study, Case 8 would have been only a grade II on this scale.

Bayly *et al.* (1984) suggested that the cause of exercise intolerance associated with ILH may be due to an increased oxygen cost of breathing resulting in diversion of blood flow to respiratory muscles and away from locomotor muscles. King *et al.* (1994) however, suggest that the condition's effects probably result from the reduction in peak  $\dot{V}O_2$ . Although they found no significant change in  $P_{aO_2}$  associated with ILH, they pointed out that both  $P_{aO_2}$  and minimal arterial oxygen saturation were several percent lower in their affected horses.

During C12, Case 8 showed no change in respiratory function as measured by the variables used in this study. Given the constraints of sensitivity discussed above, it would

be worthwhile carrying out further investigations on a group of horses with naturally-occurring ILH at the level seen here to determine whether this finding is a common one and if so whether ventilation is maintained at the cost of an increase in the work of breathing.

The fact that horses with total paralysis are still able to exercise at quite high speeds suggests that the glottis too has a significant functional reserve. If this is so, it may be that performing surgery on horses with mild to moderate ILH, merely because of the presence of inspiratory noise and disappointing performance could be a fruitless exercise. In view of the relatively high level of anaesthetic mortality in the horse, estimated at over 1% by Young and Taylor (1993), this is worthy of investigation.

Two horses, Cases 10 and 11, were diagnosed as showing dorsal displacement of the soft palate (DDSP) during fast exercise. Both horses showed a reduced  $f_b$  and increases in  $t_i$  and  $t_e$ , suggestive of loss of 1:1 respiratory:locomotory synchrony. In Case 10 there were periods of probable 1:2 synchrony, but Case 11 maintained a steady  $f_b$  throughout the canter, albeit at a slower rate than stride frequency. Whilst both horses maintained a normal  $P_{aO_2}$  during C12 (again given the wide range for this parameter), the inability to maintain 1:1 respiratory:locomotory synchrony due to the increased time required per breath suggests that any mechanical advantage of such synchrony is lost and that the work of breathing is likely to be increased.

Prior to the advent of exercising videoendoscopy, DDSP was thought to cause an immediate temporary asphyxia resulting in immediate and dramatic loss of speed. This



more sub-acute form, in which exercise continues with a milder degree of impairment appears therefore to be more subtle in effect.

The final horse with upper airway obstruction, Case 9 was tentatively diagnosed as having a congenitally small upper airway. Although cases of deformities of the upper airways have been reported involving thickening or deviation of the nasal septum of either traumatic or congenital origin or secondary to a respiratory infection (Tulleners and Raker, 1983), previously reported cases were more severe and this more subtle type of general narrowing with a normal morphology does not appear to have been reported.

Since this was one of the horses in which ventilatory data was lost, the only evidence for impairment to breathing comes from the severe reduction in proportion of normal breaths during C12 (and C10) due to interspersing of long (often biphasic inspiratory) and short breaths as the horse alternated between 1:1 and 1:2 respiratory:locomotory synchrony. Whilst this is insufficient evidence for a firm diagnosis, it is perhaps worth considering the possibility of this condition in animals in which passage of an endoscope into the pharynx is difficult with no obvious obstruction.

Post-race collapse occurs infrequently in Thoroughbred racehorses, figures for its precise incidence do not appear to be available, indeed most of the information on the condition is anecdotal. Typically affected horses stagger and may become recumbent following races, but usually recover within a few minutes. The condition does not appear to be associated with poor performance and often affected animals are seen in the winner's enclosure. Although affected animals are often given oxygen by means of a face mask or

intra-nasal tube, there is no evidence for the efficacy of this or for the presence of post-exercise hypoxaemia in these animals. The likelihood of the condition being due to hypoxaemia would seem low since following strenuous exercise the hyperventilation associated with exercise-induced hyperthermia and acidosis results in  $P_{aO_2}$  values significantly greater than at rest (Butler *et al.*, 1993a). Other possible causes for the condition include hyperthermia, acidosis and electrolyte disturbances.

Of the two Group C horses, one (Case 13) showed a slight reduction in  $V_t$  and a reduction in PEF during C12, the other (Case 12) showed no reduction in any variable, indeed PIF and  $P_{aO_2}$  were above the reference range. Neither horse showed any alterations of blood pH or mixed venous temperature when compared to the control group.

Horse number 13 was found, by examination of electrolyte creatinine clearance ratios (Blackmore and Brobst, 1981) to have abnormalities of magnesium and phosphate excretion. Dietary alterations were suggested for correction of this electrolyte imbalance. The horse is known to have won races following his return home but no follow up information was available concerning his post-race behaviour.

No consistent features suggestive of the cause of post-race problems were noted in these two horses. In view of the alarming nature of this condition, there is a need for further studies of its cause and possible therapy.

## CHAPTER TWELVE - GENERAL DISCUSSION

The preceding Chapters Four to Eleven have described the studies undertaken for this thesis. These have involved validation of the methods used, measurement of the respiratory responses of English Thoroughbred horses to exercise in a standardised treadmill exercise test and the application of that test to study the effects on respiratory function of training, influenza challenge and naturally-occurring respiratory disorders. Each Chapter has been accompanied by a discussion of the experiments described in it and it remains now to give a general discussion of the results and how they relate to the use of exercise testing in a practical context.

Since the systems used for measurement of respiratory flow rates and gas concentrations had not been previously validated it was necessary to ascertain that they are appropriate for use in the exercising horse. The data presented in Chapter Four would appear to indicate that they are capable of dealing with the high gas flows and respiratory rate generally encountered in the exercising Thoroughbred.

In only one horse in one canter was the range of the flow transducers exceeded (Monty during the C12+3<sup>1</sup> canter when fully-trained) when the right transducer inverted during peak expiratory flow. Inversions of this type indicate that the transducer has gone over-range (A.J. Woakes - personal communication). The flow transducers were therefore just able to cope with the demands of these studies, but for studies in which work loads

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<sup>1</sup>It will be recalled that C12+3 indicates a work load of twelve m.s<sup>-1</sup> on a three degree incline.

equivalent to C12+3 or greater were required it would be necessary to adjust the sensitivity of the transducers to enable them to cope with a higher range to ensure that peak expiratory flow was not lost (it is unlikely that PEF will exceed the current range by very much however).

The use of any system involving a mask for measurement of respiration is likely itself to have an effect on respiration and it is important that these effects are minimised. Harkins (1992) suggested that masks may have an effect on the work of breathing, may increase dead space or may have psychological effects. He suggested that the latter was unlikely to be important in the horse.

In the current study, however, seven of the referred horses, with less experience of running whilst wearing a mask than the experimental animals, showed more variation in the pattern of breathing at the lowest canter speed than at the highest. Since pathological effects on the pattern of breathing are more likely to be maintained as workload increases following their appearance, it appears that at low or moderate levels of exercise psychological factors may be of importance, particularly in horses less used to exercise testing. The performance and interpretation of exercise tests should take this factor into account when these circumstances apply.

It appears that the effects of this system in terms of resistance to breathing and dead space compare favourably with other systems (see Chapter Four for a fuller discussion).

The computer system for data analysis worked well in general, the one drawback being that on occasion it was unable to align the flow transducer and mass spectrometer signals accurately. This is tested for on each analysed sub-file since the mass spectrometer delay times calculated for each breath are displayed on the screen. If these times are unacceptable, it is possible to 'force' alignment using a constant delay time, specified by the operator. Unfortunately, since the true delay varies from breath to breath, the use of fixed delay times introduces a further source of error to calculations. In these studies it was found that the use of fixed delay times was too unreliable and so if the programme was unable to calculate the delay satisfactorily, it was necessary to discard the mass spectrometer data for that sub-file. In order to improve the reliability of the system for measurement of oxygen consumption, it will be necessary for refinements to be made in mass spectrometer delay calculation.

Investigation of the kinetics of the respiratory response to exercise (Chapter Six) indicated that the measured variables (excluding blood gases and pH which were sampled only twice per canter) had all reached a steady state after a minute during C8, by ninety seconds in C10 and, with odd exceptions<sup>2</sup>, by ninety and eighty seconds respectively during C12 and C12+3. Thus data obtained during the last twenty seconds of canter were considered acceptable for use in clinical testing.

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<sup>2</sup> $\dot{V}_{CO_2}$  for one horse during C12 and  $P_{E}CO_2$  for one horse during C12+3. One horse showed a slight increase in PEF throughout C12, although a plateau appeared to have been more or less reached by 80 seconds.

Training appeared to have little effect on the studied respiratory variables (Chapter Eight), with the exceptions of  $P_{aO_2}$  and  $pH_a$ , and of PIF and PEF at lower workloads ( $<10 \text{ m.s}^{-1}$ ). Thus it appears that in a mixed group of clinical referrals, the effects of differing stages of training should be small, particularly during high intensity testing. Although  $P_{aO_2}$  was affected by training it may be that this variable shows considerable between individual variation in any case and it may be of limited value for one off testing.

Qualitative assessment of individual breath flow pattern revealed unsurprisingly that in normal horses during canter the pattern of breathing is relatively constant. Individual breath typing in this way does not appear to have been used previously in the horse as a diagnostic parameter.

In the clinical group of horses (Chapter Eleven) five out of eleven animals with respiratory conditions lay outside the reference range for proportion of normal breaths, although one of the control group was also an outlier in this respect. Based on this small number of horses, this would give a diagnostic sensitivity of 44% and a specificity of 83%. Although this degree of sensitivity would be unacceptable for horses subjected to the time and expense of treadmill testing, it may be of value as a rough screening test in the field.

Animals suspected of having respiratory disease could be exercised at high workloads wearing a mask equipped with flow transducers and data logging equipment. Although exact workload would be unknown, confounding the use of *quantitative* measures of respiratory function, animals showing an abnormal pattern of breathing could be selected for further investigation. It may be that by subjecting the pattern of individual breaths to

more detailed analysis and by measuring the proportion of specified abnormal breath types, the sensitivity of this field test could be improved. Examination of individual breaths measured by telemetry during maximal ridden exercise may provide data analogous to the maximal flow volume loop which in man shows distinct patterns associated with various types of chest disease (Denison, 1994). It would be worthwhile to evaluate the diagnostic usefulness of such data in the field.

The effects of influenza challenge on respiration during exercise was investigated in eight horses (Chapters Nine and Ten). When the horses were considered as a group, no residual effect of influenza infection could be detected twenty one days after challenge. In two horses changes were detected in the pattern of breathing following challenge (although in one horse this was manifest at the two lowest canter speeds only).

One other horse showed an altered blood lactate and acid base response to exercise which appeared to be of peripheral origin. Although no sign of lameness was seen in this horse this finding is interesting in view of reports of post-viral equine rhabdomyolysis (Harris, 1990) since although myalgia is a well-reported sign of human influenza infection, no peripheral metabolic effects of the disease appear to have been reported previously in the horse. Exercise testing of horses suffering from post-viral equine rhabdomyolysis-like symptoms would be worthwhile to determine whether the response seen here represents a mild form or a precursor of this condition.

Since repeat measurements of pulmonary function on human patients following respiratory viral infections have detected abnormalities of peripheral airway function (Gibson, 1984)

it was reasonable to look for such effects in the horse as a possible explanation of the so-called 'post-viral poor performance syndrome' reported by previous authors (Mumford and Rossdale, 1980).

In fact the horses studied, with the exceptions detailed above, showed no change in respiratory function at the twenty one day stage. Possibly the incidence of horses who truly fail to recover from uncomplicated influenza (at least at the level of disease seen in the partially-immune vaccinated Thoroughbred population in this country) is too low for studies such as the present one to detect due to the small number of animals studied. However, it should be remembered that the level of disease induced in these studies was very mild due to the partial immunity of the horses and more severe episodes of influenza may have a more profound effect on respiratory function.

Since horses could not be exercised in the early part of the disease due to the danger of spreading infection to the general horse population, it was not possible to measure the effects of the acute phase of influenza on respiratory function during exercise. Such studies are undoubtedly worthwhile but for the present must await the development of exercise testing facilities in an isolation facility. A further consideration is the effect of continued training during the course of infection prior to its diagnosis. This undoubtedly occurs in horses in training, but again controlled studies of its effects are not possible without testing and training facilities in isolation.

In contrast to the influenza group, the horses with naturally-occurring respiratory disorders (Chapter Eleven) did as a group show differences in ventilation to the control group.  $f_b$ ,  $\dot{V}_c$



and PEF were significantly lower and  $t_i$  and  $t_i/t_e$  significantly higher for the referred group compared with the control animals.

In man, the wide range of many pulmonary function tests means that they are often poor at detecting lung disease on a one off test (Denison, 1994). Since the reproducibility of tests in individuals is much greater however, pulmonary function tests may be used to set an individual baseline for comparison on a later occasion (Denison, 1994).

The results of the studies on the referred horses similarly demonstrates that exercising pulmonary function tests may not be highly sensitive for the detection of lower airway inflammation on a single examination. It remains to be seen whether this is because ventilation is impaired but the variability of the tests means that they are not sensitive enough to detect it, because ventilation is maintained but at the cost of an increased work of breathing or whether low-grade inflammation may be detectable on tracheal wash aspiration at a level which does not impair ventilatory capacity.

If the latter case applies the further question must then be asked as to whether this degree of airway inflammation is insignificant or whether the inflammatory process is associated with discomfort which renders the horse disinclined to maintain ventilation during racing even though its maintenance is possible. Studies of the work of breathing in horses with upper and lower respiratory disorders may help to answer some of these questions.

Since it may not be possible to demonstrate alterations in respiratory function on a single exercise test, is there a place for treadmill exercise testing in the evaluation of the equine

patient? It is the author's belief that there is. Respiratory function testing will enable the functional effects of more severe cases to be determined and this may be useful in assessing the response to treatment by repeat testing. With further studies it may be possible to develop prognostic indicators based on pulmonary function tests, animals showing more severe changes may carry a less favourable prognosis.

Given the relatively high incidence of upper airway disorders in the Thoroughbred, a combined test in which respiratory variables are measured during initial canters, following which the horse is stopped and a videoendoscope placed in the nostril to allow visual assessment of the upper airway at high speed would facilitate the diagnosis of the presence and degree of dynamic upper airway obstructions during exercise.

Such a combined test might consist of a warm up period similar to that used in these studies, followed by a canter at eight or ten  $\text{m.s}^{-1}$ , and then a two minute canter at twelve  $\text{m.s}^{-1}$  on a three degree incline to assess respiration during the highest possible workload, at which level it is likely that psychological effects will be removed and peak flow rates will be independent of state of training.

This highest exercise level was not used for referred horses in these studies since when they began it was uncertain whether they would be safe for referred animals unused to treadmill exercise. The final canter for videoendoscopy would also be performed at twelve  $\text{m.s}^{-1}$  on a three degree incline with the canter continuing until a diagnosis was reached or the horse could no longer maintain speed. This latter regime is now in use for exercising videoendoscopy in this laboratory.

The use of treadmill exercise testing clinically is further justified by the ability to measure function in other organ systems during exercise, in particular the recording of exercising electrocardiographs and blood volume measurement would be clinically useful, whilst a strenuous standardised test allows measurement of serum muscle enzymes following testing to check for low-grade myopathies.

A further application of exercise testing is the performance of routine screening. In man such screening is used in certain populations, e.g. divers (Denison, 1994) and the good within individual repeatability of tests increases the diagnostic sensitivity of testing under such conditions. In view of the value of Thoroughbred racehorses and the cost of training them, it would be of value to carry out a periodic routine screening of horses, at the beginning of each season, for example to detect any abnormalities before full training commences. The use of serial testing in this way is already carried out by some trainers for haematology, clinical chemistry and tracheal wash sampling. Serial exercise testing may have a major role to play in equine health monitoring for the performance horse.

In summary, this thesis has sought develop a method for the measurement of respiratory function in the Thoroughbred horse and to assess its usefulness clinically. A safe, accurate method has been achieved and applied to the effects of training, experimental influenza challenge and examination of clinical cases.

The effects of training on respiratory function were found to be slight. Uncomplicated equine influenza infection of partially-immune animals was found to leave little effect on respiratory function after 21 days, but in individuals it can have peripheral metabolic effects.

Although respiratory disorders affect respiratory function during exercise, the variability of results gained from pulmonary function testing currently limits the sensitivity of a one-off test diagnostically. Serial testing of horses in training would increase the sensitivity of testing in individuals and allow for periodic monitoring of performance horses. For experimental studies, where serial testing is possible, exercise testing has a considerable role to play in the investigation of equine respiratory function and its modification by disease.

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